# Mesenchymal Diseases in Childhood

Report of the Twenty second Ross PEDIATRIC RESEARCH CONFERENCE

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The Twenty second Ross Pediatric Research Confer ence a symposium on mesenchymal diseases in child hood was held under the au pices of the Department of Pediatrics of the University of Utah College of Medi cine at Salt Lake City Utah October 25 26 1956

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## Contents

# Introduction

In the mesenchymal diseases often referred to as collagen diseases the diffuseness of our knowledge of etiology and pathogenesis has long hindered our efforts to arrive at satisfactory classification and effective treatment The search for clarification has led down many blind alleys, and doubtless many more will be traversed

These proceedings discuss current concepts through which we approach these conditions Recently developed techniques have enabled us to interrelate earlier findings with data derived from the application of these techniques raising new problems as well as laying to rest some of the old Classification has been made easier and with increased understanding of the natural course of mesenchymal diseases treatment has been made more effective

I am sure that the pooling of information among the many investigators that have been brought together here will increase the effectiveness of all in research and in clinical application

*Vincent C Kelley*
Chairman

# Role of Infection in the Etiology of Mesenchymal Diseases

DR CHANDLER A STETSON The etiology of rheumatic fever has variously been explained by virus infection nutritional deficiency and other theoric that now seem bizarre The association between rheumatic fever and infection with the hemolytic treptococcus was so evident by the 1920s however that continued research in that direction has eventually led to our present knowledge of etiology The pathogenesis of rheumatic fever may well remain obscure until the disease is successfully reproduced in animal but it seems certain that present studies are aided enormously by our knowledge that the group A beta hemolytic streptococcus is somehow the etiologic agent From this knowledge have also come the therapeutic and prophylactic measures by which we can now effectively prevent rheumatic fever and its recurrences

With the demonstration that only certain serologic types of treptococci especially type 12 cause acute glomerulonephritis the etiology of this disease also seem to be satisfactorily established It is unlikely that we shall solve the problem of the pathogenesis of other mesenchymal diseases or find effective measures for controling them until their etiology is known

*Experimental polyarthritis*

Clinicians and pathologists were once ready to accept as a working approach an infectious etiology in rheumatoid arthritis There was apparently no difficulty in reproducing analogous disease in experimental animal A high proportion of rabbits developed chronic polyarthritis after intravenous inoculation with treptococci Chronic polyarthritis clinically and pathologically much like rheumatoid arthritis, was produced by infecting mice with pleuropneumonia like organisms The presence of elevated titers of streptococcal agglutinins in the serum and/or fluid of the joints of patients with rheumatoid arthritis was repeatedly demonstrated

Subsequently experimental polyarthritis was shown to differ from the clinical disease in important respects The treptococci or

pleuropneumonia like organisms could be recovered from the infected joints in the experimental disease while cultures from joints of patients with rheumatoid arthritis were usually sterile The presence of antistreptococcal antibody seemed less significant with the discovery of the sheep cell agglutinating factor and the other peculiar properties of the serum of these patients The demonstration that the titer of antistreptolysin O was not elevated seemed to exclude the hemolytic streptococcus as the etiologic agent

Statistically valid studies did not disclose significant incidence of septic foci in patients with rheumatoid arthritis Finally the failure of antibiotics to affect the course of the disease caused the majority of workers to abandon the general notion of an infectious etiology for rheumatoid arthritis although some still clung to the possibility of a viral etiology In recent times rheumatoid arthritis has been thought to be a disease of adaptation of hyper ensitivity of collagen of the liver thyroid et cetera

Studies of the components of connective ti sue and new advances in the field of endocrinology have largely replaced the earlier bacteriologic and experimental pathologic approaches in studies of rheumatoid arthritis However de pite the advancement of several ingenious and brilliant hypotheses little real insight into the etiology seems to have emerged from either of these new lines of approach Once the etiology of rheumatoid arthritis is known the vast amount of biochemical and neuroendocrinologic information now being obtained will undoubtedly make the subsequent steps in elucidation of pathogenesis quicker and more certain

### *L forms of bacteria*

For the past 10 or 15 years a new development in bacteriology has concerned the peculiar dissociants of bacteria known as L forms Various bacteria including the hemolytic and nonhemolytic streptococci when grown under certain unfavorable conditions shed their capsules cell walls and most of their cytoplasmic constituents and exist as free living slowly reproducing organisms apparently consi ting largely of nucleoprotein The techniques of culturing the L forms are sufficiently rigorous to discourage most investigators However the basic experiments have been repeatedly confirmed and the work may well come to be regarded among the outstanding advances in microbiology of our time

The L forms are in some cases morphologically indistinguishable from the pleuropneumonia like organisms found in the human genital tract and in animals with certain diseases While there has been little tudy of the pathogenicity of L forms the extreme pecificity of pleuropneumonia like organisms for certain tissues of ome species is well known

It has been su gested that the minute pleuropneumonia like L forms would be invisible in tissues Furthermore it is likely that conditions favorable for transformation to L forms probably exi t during bacterial infection and it is possible that durin convalescence from infection the presence of antibody and other participants in the host s defenses may lead bacteria to abandon their antigenic and ulnerable cell wall and become L forms

If L forms were present in the joints it is unlikely that they would have been demonstrated by cultural techniques previou ly used L forms of streptococci are hi hly resistant to penicillin and because they do not produce streptolysin O we would not expect elevated titers of antistreptolysin O We would expect on the other hand to find antistreptococcal a lutinins in the serum of patients with rheumatoid arthritis, since these are primarily active against the nucleoproteins that make up much or most of the sub tance of the L forms

The high incidence of rheumatoid arthritis in patients with agammaglobulinemia su ge ts that the repeated infections expe rienced by these patients may have played an etiologic role and it would seem that any new appraisal of the role of infection in rheu *matoid arthritis hould include a tudy of treptococcal L forms*

## *Pathogenesis*

It seems clear on the basis of bacteriologic serolo ic and epi demiolo ic evidence that the group A streptococcus plays a causa tive role in rheumatic fever but the mechanisms by which it pro duces the rheumatic complication are as much a mystery as ever The possibility of a direct streptococcal infection of cardiac and other deep tissues remains a real one Since rheumatic fever can apparently be prevented by adequate therapy with penicillin even if this therapy is delayed for 10 days after the onset of strep tococcal pharyngitis it seems that living streptococci mu t be present in the body at the time rheumatic fever be in and not merely t o or three weeks previou ly

A concept that has received wide acceptance is that bacterial allergy or delayed hypersensitivity to streptococci is ba ic in the patho enesis of this disease When kin testing with streptococcal nucleoprotein is carried out in patients with acute streptococcal pharyngiti it is found that the great majority have positive delayed type kin reactions three or four eeks after the onset of the disea e There seems to be no correlation between the size of the reaction in the kin and the amount of circulating gamma globulin or antistrep tolysin O and there is still no direct evidence implicating hyper sen itivity in this disease

Another possibility in the pathogenesis of rheumatic fever is that endotoxins are somehow involved It has been demonstrated that bacterial endotoxins are capable of producing damage in tissues resembling that seen in certain of the mesenchymal diseases but until recently it was believed that only gram negative bacterial species elaborate endotoxins We have found however that group A streptococci contain an analogous material that produces in normal animals all the effects hitherto demonstrated for gram negative bacterial endotoxins including the production of intravascular fibrinoid It is not yet known whether the biologic activity of this material is involved in any of the nonsuppurative complications of human streptococcal infections but this is a challenging possibility that would seem to deserve exploration

As with rheumatic fever the pathogenesis of acute glomerulonephritis is still obscure The search for some nephrotoxin produced by the type 12 streptococcus seems to be a chief line of approach Another deals with the possibility that lysis by bacteriophage may occur with sudden dumping of large amounts of antigenic material into the body Hypersensitivity of either the circulating antibody type or of the delayed type may be involved but there is no direct evidence for this

In summary then the role of infection in mesenchymal disease seems well established in at least two entities acute rheumatic fever and acute glomerulonephritis and it seems possible that further study might establish its importance in others

## Role of Allergy in Mesenchymal Diseases

### *General consideration of allergic responses*

Dr William E Ehrich Allergic responses or allergies as they occur in human beings and in animals may be considered to fall into two major groups—the immediate types and the delayed type Among the immediate or early responses are the wheal and erythema type and the Arthus reaction or anaphylactic type Anaphylactic sensitivity is transmitted by antibodies in the serum which are precipitated by antigen These antibodies are gamma globulins synthesized by plasma cells Patients who cannot produce gamma globulin because they have no plasma cells are not able to develop this hypersensitivity

Responses of the wheal and erythema type can be tran ferred to other individuals by antibodies in the erum known as kin ensi tizing antibodies or atopic reagins These antibodies differ from others in that they cannot be demonstrated by precipitation with anti en although they can be demon trated by the Prausnitz Kustner reaction They do not pa s throu h the placenta as more common antibodies do sug esting a rather large molecular wei ht It now appears that these antibodies may also be formed by patients with a amma lobulinemia

Delayed hypersen itivity is characterized by the absence of antibodies in the serum Hence it cannot be tran mitted or trans ferred by erum but it can be transferred by cell Recent reports indicate that injections with BCG or other tuberculin material pro duce this tuberculin type of sensitivity in patients with agamma globulinemia

Clinically the wheal and erythema re pon e is manifested in typical cases by the appearance of hay fever asthma hives and urticaria The anaphylactic type of allergy is characterized most often by serum sickne s while the delayed respon e is u ually obscured by the manifestations of underlyin infectious disea e However in the sen itivity produced by poison ivy the direct re ponse of this de layed type of allergy may be seen in the tis ues

It should be emphasized that these re ponses are not mutually exclusive Skin sensitizin antibodies and precipitins often occur concomitantly and clinical and morpholo ic manifestations often ug e t the presence of more than one type of allergy

### *Morphologic reactions in tissues in mesenchymal diseases*

Morpholo ically the reactions in the tissues in the various me enchymal diseases are very much the same For in tance in mild or acute cases there may be mucoid de eneration (figure 1 upper left) that is swelling of the connective ti sue due to accumulation of ground sub tance Serous inflammation (fi ure 1 upper right) too may occur in acute stages This differs from mucoid degenera tion by accumulation in the ti ue of an edema fluid containing rather high concentrations of protein includin enzymes and enzymatic activators The e may cause ly is of elements of the connective tissue called de molysis

If the lesion i of greater severity lar er molecule such as fibrinogen pass throu h the capillary wall and are precipitated in the connective ti sue in the form of fibrinoid a process known as fibrinoid de eneration (figure 1 lower left) Fibrinoid apparently differs from fibrin in that the precipitate includes other ub tances

along with fibrinogen which can be demonstrated in tissues with ordinary histologic techniques There may be other proteins involved and carbohydrates fats and even nucleic acids may be precipitated Fibrinoid is not a uniform substance and it is clear that there are various fibrinoids all of which probably contain fibrinogen

In the most severe cases the deposition of fibrinoid may be associated with necrosis referred to as fibrinoid necrosis If the collagen disease is chronic we usually do not see retrograde or



*Figure 1* Morphologic reactions in tissues characteristic of mesenchymal diseases (upper left) mucoid degeneration (upper right) serous inflammation (lower left) *fibrinoid degeneration and (lower right) granulomatosis* Reprinted from The Journal of Experimental Medicine *89* 3 (January) 1949

regressive changes but rather productive or proliferative changes known as granulomas (figure 1 lower right) Some of the granulomas that develop in diseases of connective tissues are highly specific The Aschoff bodies for example are so characteristic that they are diagnostic of the disease However in many other cases the granulomas are non specific

The wheal and erythema response is characterized by serous inflammation The vessels become extremely permeable and components of plasma leak into the tissue On the other hand the Arthus or anaphylactic response is characterized by severe fibrinoid degeneration and necrosis especially of the arteries while the delayed response is characterized by the formation of granulomas

The outcome of the various reactions that we see in the collagen diseases is quite uniform Mucoid degeneration may resolve spon taneously without leaving any scar or other evidence of existing di ease On the other hand serous inflammation fibrinoid degen eration and necro is and granulomato is re ult in sclerosis or scar *formation with permanent damage*

The involvement of the connective tissue in these various diseases is frequently a ociated with similar reactions of the vessels more frequently the arteries suggesting that the noxious agent was pre ent in the circulating blood and entered from there into the arterial wall Thrombosis is commonly observed in the vessels and the com position of the thrombi appears to be as variable as the compo ition of the fibrinoid

Ten to twenty days after the fir t *injection* of large doses of horse serum proliferative changes d velop in the *vascular connective* tissue with swelling of the musculature and proliferation but not degeneration of cells If the injection is repeated a udden clash of large quantities of antigen and antibody in or on the vascular connective tissue results in fibrinoid necrosis In many cases in which proliferative lesions develop from the first injection degenerative lesions appear after the second injection

## *Allergic factors in pathogenesis of specific mesenchymal diseases*

The changes in the tissues characteristic of collagen disease are generally identical to tho e *seen in serum sickness* Although *serum* ickness is known to be an allergic response the collagen diseases are not nece sarily of allergic nature Fibrinoid degenera tion and perhaps other changes *seen* in these diseases can be elicited by other mechanisms However the lesions in the so called collagen diseases are consi tent with the po ibility of allergic origin

A di ea e resembling rheumatic fever has been produced ex perimentally by the induction of successive infection with different types of group A hemolytic streptococci The fact that the erum of mo t patients with rheumatoid arthritis stron ly a lutinate group A hemolytic streptococci and sen itized sheep cells has been *offered as evidence that rheumatoid arthritis is a product of hyper* ensitivity However ome investigators have reported that the agglutinins *or* reactors in these patients differ from antibodies in various ways They cannot be uppressed by cortisone as are other antibodies and furthermore it has b en hown that nonsensitized colloidal particles like ise are agglutinated by these ra I wonder whether these are not *non pecific in tabilities that occur in the* disease in the colloid chemical tatu of the serum an l maybe also of

*the connective tissues Surely there is no direct evidence to prove that* these instabilities are caused by true antigen antibody reactions or any other allergic mechanisms we know of

There is no evidence as yet that systemic lupus erythematosus dermatomyositis or scleroderma are of allergic origin On the contrary there has been a recent report of generalized scleroderma in a child with agammaglobulinemia

*Polyarteritis nodosa and serum sickness resemble each other* so closely that they may be considered different expressions of the same process An antigen antibody reaction of the anaphylactic type seems to be involved However patients with the former disease frequently develop severe asthma a manifestation of the wheal and erythema type of allergy

We can conclude then that in some of the diseases of connective tissue *an allergic nature has been definitely established In* others certain observations suggest that allergy may be a factor while in a third group at least the anaphylactic type of allergy can be definitely excluded

## Discussion

### *Development of mesenchymal diseases in patients with agammaglobulinemia*

Dr Robert A Good Ehrich mentioned that we might learn something concerning the possible allergic etiology of mesenchymal diseases from interpretation of the experiments of nature presented by patients with agammaglobulinemia These patients have a gross deficit in capacity to form circulating gamma globulin and have almost no circulating antibodies We have observed two patients *with agammaglobulinemia who have developed* classic atopic dermatitis a sensitivity reaction dependent on the nonprecipitable wheal and erythema type antibody Recently I learned of a patient with agammaglobulinemia who had hay fever Circulating nonprecipitable heat labile antibody was identified in significant concentration *and* this sensitivity could be transferred by injection of serum to a non sensitive recipient

We have observed four patients with agammaglobulinemia who have developed typical rheumatoid arthritis The incidence of rheumatoid arthritis in our series of patients with agammaglobulinemia is therefore considerably greater than the incidence in the population *at large Hansen studied a patient with congenital agammaglobu*linemia failure of the immune response who developed classical dermatomyositis

Patients with agammaglobulinemia can develop tuberculin reaction and can react to administration of typhoid paratyphoid antigen as do normal patients. Bacterial allergy against streptococcal products can be transferred to patients with agammaglobulinemia and they are able to sustain this form of allergy. We have been able to induce in patients with agammaglobulinemia delayed allergic responses to certain chemicals and to transfer this delayed allergy to subject who have not had contact with the chemical.

It seems we should be less concerned with classic circulating antibodies as important pathogenetic agents in this group of disease and more concerned with the delayed allergy or possibly the allergy dependent on the heat labile nonprecipitating reagin type of antibody.

DR STETSON Because patients with agammaglobulinemia are extensively and energetically investigated I wonder whether some of the things done to them perhaps contributed in some way to the rather high incidence of mesenchymal and mesenchymoid diseases.

DR R A GOOD Fortunately for us with our intensive investigation three of the four patients with agammaglobulinemia who have had rheumatoid arthritis had the disease at least two years before any studies were carried out.

# Psychosomatic Aspects of Mesenchymal Disease

DR C KNIGHT ALDRICH In the development of any psychosomatic illness emotions participate as one of a group of causative elements which vary in significance as well as in response to treatment from individual to individual.

### *Emotional factors in the etiology of rheumatoid arthritis*

The interrelation of emotional factor with other contributing causes in the etiology of rheumatoid arthritis in children is complex. Other participating factors are assumed to be of hormonal inherited traumatic or infectious nature. Personality characteristics adaptation pattern and historic event similar to those found in the lives of patients with rheumatoid arthritis have been observed in patients with other psychosomatic disorders certainly suggesting that emotional factor are not the only important determinants of

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the site of symptoms Investigations of the significance of emotional factors are handicapped however by the chronicity and severity of the illness as well as by the lengthy and time consuming nature of psychiatric treatment Thus it is difficult to differentiate the effects of treatment from the effects of events in the natural course of the patient s life and it is difficult for any investigator to treat a statistically significant number of patients

In spite of these difficulties there is some evidence that emotional factors make both direct and indirect contributions to the development of rheumatoid arthritis, and some indication that similar factors affect other diseases of the mesenchymal group The evidence accumulated by studying developmental histories and the personality structure of adults with rheumatoid arthritis points to a characteristic constellation of personality factors These studies demonstrate persistent and powerful but unfilled dependent needs arising out of early disturbances in the mother child relationship resulting in hostility towards the mother which is ordinarily channeled into a masochistic need to serve others In other word the arthritic patient in the earliest stage of development has lost out on some of the gratifications he should have received from the dependent relationship with his mother He therefore remains chronically insecure having stored up the child s equivalent of intense anger towards the mother who failed him His fear of losing the small security he has prevents his expressing hostility directly He learns that to serve others wins their approval and keeps his hostile feelings under control

Sometimes however situations arise that either interfere with masochistic methods of dealing with hostile feelings or else mobilize guilt secondary to the hostility These situations are associated in predisposed individuals with the onset or exacerbations of rheumatoid arthritis

Electromoygraphic studies have provided suggestive evidence that the patient with rheumatoid arthritis is likely to reflect his psychologic tensions through somatic muscular tension This evidence preliminary though it is supports the theory that persistent increased muscle tonus resulting from inhibited aggressiveness and the defenses against it in some way precipitates the attack of arthritis Glaser has noted that when the emotional tension of patients with rheumatoid arthritis finds redirection in manifestations of psychosis the arthritic symptoms remain minimal The alternation of psychosis and psychosomatic symptoms indicate the interrelationship of emotions and this type of symptom

Surveying the results of their psychotherapy on the arthritic process in children Blom and Nicholls report their impression that with treatment remissions come sooner and last longer but state that

improvement can be better demonstrated in terms of general emo tional adjustment than in alteration of arthritic symptoms

### *Emotional effects of mesenchymal diseases in children*

The emotional effects of me enchymal diseases in children can be discussed under three general headings emotional effects of any chronic disease implications of changes in the child s body image emotional by products of treatment particularly of treatment with steroid hormones

Any disease that produces chronic invalidism subjects the pa tient to an unusual degree of dependency on his environment The helples ness and restrictions imposed by illness may produce a va riety of emotional reactions Some children become particularly anxious others are depressed apathetic or withdrawn Still others deny their incapacity thus avoiding the necessity to face their de pendency The choice of reaction by a given child depends on the um total of the relationships experienced and the attitudes developed in the earliest period of his life when dependency was inevitable

When nece sary mature adults can accept the dependency of illness with equanimity remaining eager and willing to give it up when their physical condition improves This process is more diffi cult in children and immature adults Early experiences have con ditioned some to equate helplessness and dependency with abandon ment or rejection others have been conditioned to feel ashamed of any dependent yearnings These attitudes contribute to the devel opment of anxiety and depression during illness of any kind Other children appear to be trying to capture a security they did not re ceive when they were younger accepting illness reasonably well but resisting convalescence and seeking to maintain their dependent position

The distortion of body parts brought on by illness is frighten ing enough to an adult but much more so to the child The child who sees a joint becoming swollen painful and stiff does not easily comprehend that the illne is likely to be limited to the joints but may have terrifying fantasies that the disability will in some way extend to other more vital parts

The patient s concept of body image is important in the re action to treatment, particularly by corticoid It is difficult to estab lish in what proportion the biochemical effects of th corticoids and the anxieties associated with the modifications in body image con tribute to the psycho es occasionally een during treatment with these substances The confusion in identity and the distortion of body image produced when an adolescent girl finds her face changing in shape

accompanied by the development of extensive acneform lesions and hirsutism may be more significant in producing a psychosis than the alterations in body chemistry. Assuming that both play a part, it is particularly important in the interest of mental hygiene to provide constant reassurance, explanation and interpretation of the nature of the disease and the side effects of treatment to any child with mesenchymal disease.

**Biochemical and Hematologic Alterations in Mesenchymal Disease**

# Some Chemical and Biologic Properties of Chondroitin-sulfuric Acid B

DR ALBERT DORFMAN In recent years many diseases have been thought to be associated with changes in ground substance Because of the limited histochemical methods available interpretation of such changes has been unsatisfactory

There is now sufficient evidence to indicate that ground substance is not a uniform chemical substance but rather a complex physicochemical or colloidal solution This amorphous material separates fibers and cells and is thus the phase interspersed between all parenchymal cells and circulation The ground substance contains materials in transit between cells and circulation and substances peculiar to connective tissue The composition of ground substance varies in different parts of the body and in different tissues In cartilage synovial fluid and vitreous humor for example the composition appears to be considerably simpler than in skin

### *Proteins of ground substance*

The proteins of ground substance include acid soluble collagen neutral soluble collagen protein components of mucopolysaccharide complexes and possibly precursors of elastin

The existence in vivo of acid soluble collagen in solution remains questionable It is apparent from recent work that a soluble form of collagen called the neutral soluble fraction is present in large quantities in ground substance and has a rapid metabolic turnover

Sulfated mucopolysaccharides exist in certain tissues as protein complexes of high molecular weight The chemical linkage between protein and mucopolysaccharide is unknown It seems likely that precursors of elastin should also be present in the ground substance

obtained from the unknown compound behaved on paper chromatography just as that obtained from a known sample of *l* iduronic acid

It is of interest that iduronic acid has never been demonstrated to occur naturally *D* glucuronic acid and *l* iduronic acid differ only in the stereoisomerism of the fifth carbon atom

## *Some biologic properties of chondroitin sulfuric acid B*

Another approach to the study of this compound has concerned its biologic activity Marbet and Winterstein have reported that the antithrombic activity of chondroitin sulfuric acid B is considerably less than that of heparin However the former compound occurs in connective tissue in rather large amounts 25 mg of the pure compound can be isolated from the skin of one rabbit but only minute amounts of heparin can be isolated from tissues Even if the anticoagulant activity of chondroitin sulfuric acid B is weak compared to that of heparin the total activity might be great Chondroitin sulfuric acid A demonstrates no antithrombic activity

For study of relative antithrombic activity the basic design of the experiments carried out with Grossman consisted of incubating a mixture of purified thrombin with the particular compound in question and in most cases with plasma After a period of incubation of 15 minutes an aliquot was mixed with fibrinogen and the clotting time determined Increase in clotting time beyond that of the control indicates antithrombic activity

It has long been known that heparin alone is not an antithrombic substance but requires for activity some other component present in plasma There has been considerable disagreement on whether this component should be called a heparin cofactor or whether heparin itself should be considered the cofactor and the component in plasma the antithrombic substance

With impure fibrinogen heparin appears to be active because of contamination with antithrombic substance For this reason we utilized purified fibrinogen Under these conditions chondroitin sulfuric acid B alone does not demonstrate antithrombic activity but when mixed with plasma the antithrombic activity of plasma is augmented In the presence of plasma and at low concentrations of thrombin (10 to 20 units/ml) chondroitin sulfuric acid B has considerably more antithrombic activity than heparin However at high concentrations of thrombin (200 units/ml) chondroitin sulfuric acid B does not exhibit antithrombic activity

Figure 3 represents the results of an experiment indicating the relative antithrombic properties of plasma of plasma plus heparin and of plasma plus chondroitin sulfuric acid B All clotting assays were performed at theoretically constant thrombin concentrations

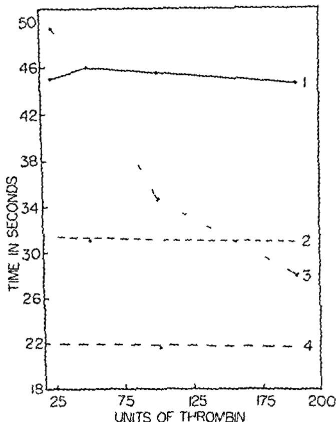



*Figure 3* The relative nt thromb a t ity of pla ma pla ma plus heparin and plasma plus ch ndro t n ulfur a d B after ubat on with ari us co centrat ons of thromb n The aliq iots of the i cubat on mixt w e d luted bef re react on with fibrin g n so that in ea h case the same amount of thrombin was used n determin ng the o ulat on tim Curve 4 represents the c agulat on of the c nt ol s m les thromb n plus fibri n gen Cur e ndicates th effect of incub t ng the th ombin w th plasma and Curve 1 ind ates the ffect of n ubatn n of thromb n plasma and hepa n In these thr e urs s t may be seen that the concentration of thromb n n th incubat on mixture does not appear to infl e nc the result However wh n thr mb n pla ma and hon } t sulfur acid B are n ubat d togethe th ant thromb a ti ty (Curve 3) great when th onc ntrat on of thromb n in the in ub t n mixt re is l w a d a tually less than that of pla ma al e wh n th on e trat n of th omb n n th ncubation mixtu e h gh

but the thrombin concentration was varied in the thrombin plasma polysaccharide mixture Plasma exhibits definite antithrombic activity that is augmented by the addition of heparin at all concentrations of thrombin tested The addition of chondroitin sulfuric acid B to thrombin and plasma results in marked augmentation of antithrombic activity at low concentrations of thrombin At high concentration of thrombin chondroitin sulfuric acid B is not only less active but partially neutralizes the antithrombic activity of plasma

These data would seem to indicate that certain polysaccharides in ground substance may have highly specific activities that are quite different from those of other compounds closely related chemically We do not know the exact chemical structure of chondroitin sulfuric acid B or anything about its metabolism What role it may play in physiologic or pathologic conditions is not clear It is clear however that there is a substance normally present that could profoundly affect the deposition of fibrinogen in the ground substance of connective tissue and it seems possible that the amount of such material present its physicochemical state or its dissociation from proteins may well play some important role in both physiologic and pathologic processes

## Significance of Miscellophagosis in the Physiology of Ground Substance

DR ROBERT D HIGGINBOTHAM I should like to review some studies concerning an interrelationship of the mucopolysaccharide of ground substance with cells of connective tissue in the maintenance of the physiologic state They deal in large measure with the intracytoplasmic storage or sequestration of noxious foreign material a collaborative function of ground substance and connective tissue cells that we have called miscellophagosis Concerned in this process are the mast cells the fibroblasts and of course ground substance—the amorphous continuum between the fibers cells and vessels of the connective tissue

The importance of miscellophagosis is implied by the interrelated roles of the connective tissue elements in the transport of metabolites and the maintenance of an optimal environment for cellular function Changes in both chemical and physical states of the ground substance as well as of the cellular constituents of connective tissue are indicative of altered function and are characteristic of the inflammatory response

### *Combination of mucopolysaccharides with noxious foreign materials*

Ground substance may function in a manner analogous to that of ion exchange resins. The calcium binding properties of cartilage are well known and a selective affinity for the potassium ion has been demonstrated for the polymerized ground substance of the symphysis pubis of the guinea pig. This postulated role of ground substance as an exchanger of ionizable substances would be governed in part by the number of acidic groups present and the degree of polymerization of the mucopolysaccharides as well as by the complex nature of the other constituents of ground substance.

We have tried to determine whether the binding of various substances by mucopolysaccharides may function in the presence of noxious agents as a mechanism for the maintenance of the physiologic state in the connective tissue. A schematic illustration of this function is shown in table 2. The acidic mucopolysaccharide MPs binding with an appropriate substance X which may be an amine or protein of the connective tissue forms a complex with it. This complex in the presence of another substance Y may be dissociated forming a new complex MPs — Y and releasing X from the original complex. The substance Y would therefore be known as a releaser.

Preliminary studies have shown that a complex of heparin with toluidine blue may be dissociated by basic substances that have a strong affinity for this mucopolysaccharide. The second complex at optimal proportions forms a precipitate and the dissociated dye is freely dialyzable. The release of dye shows indirectly that the heparin has formed a new complex with the basic substance or toxic agent and thereby reduced the concentration of active toxic substance.

The relative strengths of these releasers were estimated from the amount required to release 25 to 50% of the toluidine blue from its metachromatic complex with heparin. A number of the strong and moderate releasers such as polymyxin, neomycin and the chemotherapeutic diamidine compounds are therapeutic drugs whose use has been restricted because of the toxicity associated with them. Of the other substances, both 48/80 and basic lead acetate are reportedly very efficient releasers of histamine from the heparin histamine complex in tissue.

After subcutaneous injection of heparin in the mouse metachromatic granules appeared in the local fibroblasts suggesting that the cells were able to ingest and concentrate this substance as intracellular granules. Injection of chondroitin sulfate B produced a similar although weaker effect than that of heparin.

Additional studies showed that chemically sulfated hyaluronic acid as well as chondroitin sulfate A appeared in the cells two hours

after injection The cells that ingested these chemically altered mucopolysaccharides were unable to dispose of them and granules were seen in their cytoplasm for at least one month after injection in contrast to the 24 to 48 hours required for disappearance of naturally occurring mucopolysaccharides

The sequestration of such altered or foreign material and the intracytoplasmic storage which we have termed micellophagosis suggests that the cellular function is also one of detoxification the ingested material being no longer free to act extracellularly The initial

**Table 2**

*Acidic mucopolysaccharide exchange of basic substances*

MPs + X ⟶ MPs X
MPs X + Y ⟶ MPs Y + X

Heparin Toluidine Blue (50 $\mu$g) (120 $\mu$g) + Releaser ⟶ (5 0 ml) Heparin Releaser + Toluidine Blu

Releasers

| STRONG | MODERATE | WEAK | INEFFECTIVE |
|---|---|---|---|
| 48/80 | Stilbamidine | Cadaverine | *d* tubocurarine |
| Polymyxin | Pentamidine® | Putrescine | Pyribenzamine® |
| Protamine | Propamidine® | Streptomycin | Tryptamine |
| Neomycin | Spermidine | | Morphine |
| Clupeine | Basic FbAc | | Glucosamine |
| ACTH | | | Bacitracin etc |

Compared on basis of amounts necessary for dye release (DR) STRONG = 50% DR/25 100 $\mu$g MODERATE = 50% DR/250-1000 $\mu$g WEAK = 25% DR/1000 $\mu$g and INEFFECTIVE = 25% DR/1000 $\mu$

inflammatory response subsides after a time despite the continued presence of the noxious substance in the tissues The presence of dark metachromatic granules in the cytoplasm of the fibroblasts provides an appearance quite similar to that of the mast cell We have described such cells as quasi mast cells to denote this superficial resemblance

Further investigations included subcutaneous injection into mice of mixtures of heparin with various basic substances heparin 48/80 complex heparin stilbamidine complex heparin polymyxin complex and heparin neomycin complex It was observed that these substances which reacted with heparin in vitro to form a cloudy solution altered either the appearance of the granules or the rate at which they were formed Many of the basic substances used are biologically active and

in suitable concentration induce toxic reactions culminating in the death of the experimental animal We found that pretreatment of the animal with heparin markedly enhanced its tolerance to these drugs For instance administration of 100ug of heparin proterted all of the mice challenged with a dose of 48/80 that killed all of the aline treated controls Similar protection was obtained against polymyxin neomycin and stilbamidine Conversely the prolonged coagulation time of the blood of heparinized mice could be markedly reduced by the injection of many of these toxic sub tances

The observed tolerance of the heparin treated mice appears to depend initially on the interaction of this acidic mucopolysaccharide with the toxic basic substance However the range in complexity of these toxic agents from a synthetic phenylalkylamine to a naturally occurring polypeptide makes it unlikely that a specific molecular structure of the toxic agent is required for its fixation by heparin

## *Role of the mast cell and fibroblast in micellophagosis*

Our observations would suggest that the mast cell may origi nate in the ti ues by a mechanism similar to that hown for the granulation of fibroblasts The mast cell is known to contain rela tively large amounts of biologically active substances heparin and histamine which appear to b stored in the granules of the cell Since various kinds of injury to the tissue can cause the degranula tion of mast cells and shedding of granules into the surrounding ground sub tance we have been intere ted in determining the fate of this histamine and heparin containing material

Fifteen minutes to an hour after relea e of the granules granules staining similarly can be seen in the cytoplasm of surrounding fibro blasts suggesting that the fibroblasts are able to ingest the shed granules of the mast cells When granules of mast cell isolated from the subcutaneous connective tissu of mice were injected subcuta neously into other mice many of the local fibrobla ts were sub e quently found to have their cytoplasm filled with this granular ma t rial Similar experiments employing isolated $S^3$ labeled granules of ma t cells obtained from mice previou ly treated with radioactive odium sulfate confirmed our hypothesis that the granules in the fibroblasts were those shed from the ma t cell

Ingest d granul s appeared to di solve or be metabolized within the cytoplasm of the fibrobl t implicating the fibrobla t as a par ticipant in the di po al of histamine containing material as well as in the metabolism of mucopolysaccharides of ti ue In the Arthus phenomenon the reaction involving the ma t cell is more marked and appears to overwhelm the capacity of the local fibrobla t to inge t the numerous shed granules

Dr Ehrich There have been recent reports indicating that 48/80 releases histamine from the heparin histamine complex suggesting that this material is a histamine liberator As stilbamidine adrenocorticotropin et cetera release toluidine blue I wonder whether we can assume that they are also histamine liberators?

Also I should like to ask what you think is the metabolic significance of the uptake of mucopolysaccharides by fibroblasts? Where are the mucopolysaccharides produced? Do you believe it possible that the fibroblasts take up mucopolysaccharides and convert them into hyaluronic acid?

Dr Higginbotham I think there are degrees of histamine release and 48/80 stilbamidine and polymyxin are histamine releasers both in vivo and in vitro while other substances may be active in vitro only It seems quite possible that the release occurs by replacement since the releasers combine quite strongly with heparin How the binding of histamine by heparin comes about is a subject for future work

The question concerning the turnover of the mucopolysaccharides is difficult to answer We might assume that fibroblasts are involved in production of hyaluronic acid and other mucopolysaccharides We made radioautographs of loose connective tissue at various stages after injection of radiosulfate into animals Radiosulfate has been thought to enter heparin in an exchange reaction with the sulfate of the heparin molecule However as early as six hours after injection we found a portion of the sulfate fixed in the ground substance and the amount steadily decreased during the next 48 hours Meanwhile radioactivity in the mast cell increased suggesting the possibility that heparin was being synthesized outside of the mast cell and was then accumulated in it

## Alterations of Fibrinogen and Related Proteins during Mesenchymal Disease

Dr Richard T Smith Most of our studies have been concerned with the origin and nature of the fibrinoid deposits, such as

may be seen in and around the blood vessels in systemic lupus erythematosis in the rheumatic heart and in the connective tissue in rheumatoid arthritis and related disorders Both clinical and experimental data indicate that this material is composed chiefly of fibrin or a fibrin like protein and a mucopolysaccharide The generalized Shwartzman reaction provided an experimental model for studying some aspects of the origin and composition of fibrinoid deposits in experimental animals

### *Demonstration of reversibly cold insoluble fraction*

During attempts to find a circulating precursor of fibrinoid in rabbits given intravenous bacterial endotoxin a component of heparinized plasma that precipitated on standing at 4°c was observed This material was termed a heparin precipitable fraction since it was reversibly insoluble only in the presence of heparin or related acid mucopolysaccharides This fraction like fibrinogen disappeared from the circulation with the deposition of fibrinoid material in the kidneys spleen and heart Heparinized plasma taken from children with acute rheumatic fever was also found to show a heavy protein precipitate after chilling similar in appearance to that observed in animals which also disappeared when the plasma was warmed

### *Physicochemical properties of the heparin precipitable fraction*

Material employed for studying the physicochemical properties of the fraction was the twice washed cold precipitate from the heparinized plasma of an acutely ill patient It was found that heparin chitin sulfate polymannuronic acid sulfate and certain dextran sulfates were equally effective in producing cold precipitation in the plasma of acutely ill patients in contrast to calcium binding anticoagulants Maximal precipitation occurred with the concentration of heparin at 0 1 mg/ml Increasing ionic strength between 0 02 and 0 5 increased the solubility of the fraction as did increasing pH within the range of 6 7 °4 Calcium or magnesium ions were required for maximal precipitation

Several pieces of evidence indicate the close relationship of this fraction to fibrinogen 1) its absence from heparinized serum 2) electrophoretic motility similar to that of fibrinogen 3) 80% of the fraction had sedimentation characteristics in the ultracentrifuge similar to those of fibrinogen 4) the fraction was 50 80% clottable by thrombin Th fraction differs from pure fibrinogen however in solubility when combined with heparin marked enhancement of rouleaux formation and sedimentation of erythrocytes only partial clottability and the presence up to 30% of a high molecular weight component

We are entertaining the working hypothesis that the fraction represents a combination or mixture of fibrinogen and an intermediate polymer of fibrinogen perhaps partially denatured which has an affinity for *acid mucopolysaccharides and as a consequence of combination with* these substances has the altered physicochemical properties we have described

### *Concentration of heparin precipitable fraction in serum*

Table 3 presents data on the amount of heparin precipitable fraction found in the plasma of normal individuals of various ages and table 4 summarizes the findings in various disease states High concentrations of the heparin precipitable fraction occur in the plasma of humans with acute disease whether due to infections or inflammatory or mesenchymal disease When the patient is severely ill with any of these disorders the concentration often equals or exceeds that of clottable protein The high values return to normal after successful treatment or spontaneous remission of the disease

We conclude from such studies that the occurrence of large *amounts of heparin precipitable fraction in the plasma of humans* is another of the so called acute phase phenomena The significance of this fraction is not understood However the occurrence in the circulation during acute disease of a protein with an unusual affinity for acid mucopolysaccharides, suggests a possible function in handling the mucopolysaccharides that result from tissue destruction The suggestion that the heparin precipitable fraction in humans is a fibrinoid precursor is as yet unsubstantiated but deserves further study

## Discussion

Dr Dorfman I think most chemists who work with the acid mucopolysaccharides would object to saying that because a material is Schiff positive it is an acid mucopolysaccharide Some lipids may also be Schiff positive

Dr Stetson If some of the *heparin precipitable protein is non* clottable why is none found in the serum?

Dr Smith A likely explanation of the absence of the *fraction from serum* is that *in the clotting process* of whole blood fibrinogen absorbs the heparin precipitable fraction of the plasma However it is entirely conceivable that the antithrombic effect of heparin itself might influence clottability and give falsely low values in our determinations

## Table 3

*Conceutrations of heparin precipitable fraction in plasma of normal indiuiduals*

| Age (y s) | N mb | M l | | | F m l s | | |
|---|---|---|---|---|---|---|---|
| | | No | M a | S D ± | N | M | S D ± |
| 0 15 | 139 | 69 | 0 101 | 0 043 | 0 | 0 104 | 0 042 |
| 16-45 | 1 9 | 80 | 0 179 | 0 049 | 99 | 0 111 | 00 9 |
| 45 ,5 | 188 | 85 | 01 1 | 0 051 | 103 | 0 13 | 0.0a3 |

## Table 4

*R l ilit concentrations of heparin precipitable fraction in the pl isma in various conditions*

V y h gh lly h gh th f b og
- A ute l umat fev (40)
- A t e l snato l arthrit (70)
- A ite l A terial m nin t pt m a (17)
- A t treg t o al sl syn s (6)
- A ute ur ary t t infect n ( )
- Hod k ns d a (3)
- D m n t d mal gnancy (4)
- P egnan y p t ularly v th toxem a (1,0)

U lly h gh w th f s d f b g
- Ch onc rh umatoi l a thrit s (5)
- Ch n inf t n of the lung s nu a ( 0)
- U m a (4)
- Unt eat l conv lesc nt heu mat c fever (6)

U lly w th m l l m t
- C mmon ld (3)
- Mump (3)
- H pat t s ( )

U lly l w
- N wborn infant ( 0)
- Ad nced l er d ease (5)
- P lvarteriti nodosa ( )
- Car l ac f ilu e (4)
- N pl ro s (13)

( ) ind ate numb r f pat ents studied n lly

DR S VAN CREVELD We know that a heparin neutralizing factor is present in platelets In pla ma if the platelets remain intact the factor is not present

DR SMITH We have always been concerned about the possibility of an effect exerted by some factor in platelets As a consequence we attempt to minimize the platelet effect by appropriate high speed centrifugation as soon as we obtain the blood from the patient

# Behavior of Proteolytic Enzyme Inhibitor in Serum during Inflammatory States

DR FREDERICK C MOLL In the process of inflammation the role of the proteolytic enzymes has not been defined but fibrinolysin and the proteases of leukocytes are thought to contribute to the dissolution of tissue In the allergic response in vitro the liberation of proteolytic enzymes precedes the elaboration of histamine and heparin Serum however contains inhibitory substances that inactivate the leukoproteases plasmin trypsin and chymotrypsin These inhibitory substances may be of importance in the dynamics of inflammation

The clinical studies of the proteolytic enzyme inhibitor in serum have extended over more than 50 years yet until recently it was known only that the concentration of the inhibitor in the serum is increased in pathologic conditions that are associated with destruction of tissue and the fluctuation in concentration parallels fairly well the sedimentation rate In chronic inflammatory diseases the concentration is elevated at first and returns to normal as the activity of the disease state subsides

It has recently been demonstrated that the larger fraction of the inhibitor which inhibits trypsin travel electrophoretically with the alpha globulin and a smaller fraction effective against plasmin as well as trypsin travels with the alpha$_2$ globulin and accounts for only about 10% of the total trypsin inhibitory power of serum

The method we employed for titration of the inhibitor consists of measurement of the inactivation of trypsin by serum in a casein substrate at 37°c and pH 7 6 the area in which trypsin is most active The method used was standardized against one employing the inhibitor obtained from soybeans

### *Experimental studies in concentration of proteolytic enzyme inhibitor*

As may be seen in figure 4 normal monkeys show no significant variation in the level of the serum inhibitor from day to day With the inflammation produced by a turpentine abscess there is a prompt

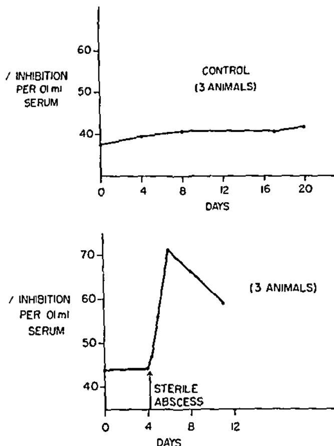
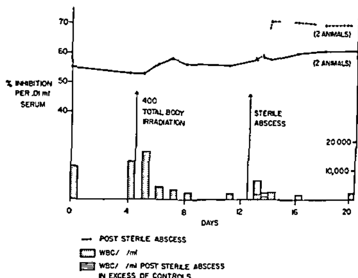



*Figure 4* Concentrations of proteolytic enzyme inhibitor in serum of monkeys under various experimental conditions (Upper) Normal control animals. Note the constancy of the concentration from day to day (Lower) After production of sterile abscess by injection of turpentine there is a prompt increase in concentration Data from original article in The Journal of Experimental Medicine

rise in the concentration of inhibitor in the serum This rise has been thought to be due to the accompanying destruction of tissue

When the entire body was irradiated to produce destruction of tissue there was a prompt and extensive decrease in the total leukocyte count but the concentration of inhibitor in the serum increased only slightly (figure 5 upper) On subsequent production



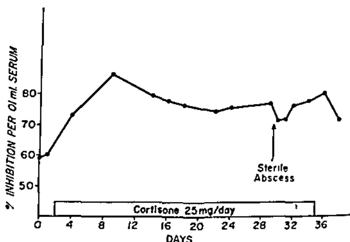




*Figure 5* Concentrations of proteolytic enzyme inhibitor in serum of monkeys under various experimental conditions (Upper) Irradiation of the entire body resulted in only slight increase in concentration of the inhibitor in the serum but subsequent production of a sterile abscess in two of the animals was accompanied by definite increase (Lower) The administration of cortisone resulted in a prompt and prolonged elevation of the concentration of inhibitor in the serum and no significant increase in the concentration occurred after the subsequent injection of turpentine Data from original article in The Journal of Experimental Medicine

of a sterile abscess by injection of turpentine a prompt rise accompanied the inflammatory reaction. When irradiated animals were subjected to electrocautery producing a lesion comparable in size to the inflammatory reaction of the turpentine abscess only a light rise in concentration of the inhibitor occurred.

In humans injection of adrenocorticotropin and exposure to cold temperatures have been shown to result in elevation of the concentration of trypsin inhibitor in the serum. The effect of administration of cortisone was therefore studied in a group of animals and a prompt rise of the inhibitor was noted (figure 5 lower).

The mechanism through which the inhibitory activity is increased by cortisone is not known. No source of the inhibitor in tissue can be demonstrated despite our attempts at assays for the inhibitor in all organs including the sites of inflammation produced by the abscess.

On the twenty eighth day of administration of cortisone an abscess was again produced by injection of turpentine. There was no significant change in concentration of inhibitor in the serum (figure 5 lower). The failure to respond to the inflammatory stimuli during administration of cortisone suggests that the animal failed to elaborate more inhibitor by the usual pathways because of suppression of adrenal activity.

## *Physiologic effects of isolated proteolytic enzyme inhibitor*

The partially purified proteolytic enzyme inhibitor was demonstrated by paper electrophoresis to be primarily an alpha globulin and to contain little if any alpha globulin. The inhibitor was nearly completely destroyed by heat at 56°c for one hour was unstable at room temperature at pH 3.6 but relatively stable at higher values of pH. The partially purified material was inactive against plasmin which would be anticipated since only the alpha inhibitor is effective against plasmin.

In further studies of this inhibitor in relation to the inflammatory process, guinea pigs were sensitized with egg albumin. Various sites of the skin were then prepared for study by injection of the partially purified inhibitor. The inhibitor injected in some of the sites had been inactivated by heat, while that injected into other sites had not been inactivated. Albumin was then injected into each one of these areas and partial suppression of the inflammatory reaction was observed in the sites of injection of the inhibitor that had not been subjected to heat.

Despite the experimental studies, the clinical significance of the trypsin inhibitor is certainly not clear. It is true the concentration of inhibitor in the serum is elevated in mesenchymal diseases

and returns to normal during remissions of the disease However as with the hyaluronidase inhibitor administration of cortisone appears to raise the concentration of the inhibitor but, when the concentration is high as in rheumatic fever treatment with cortisone is associated with a decrease in concentration and with a subsidence of the inflammatory reaction

I have postulated that these bizarre and contradictory findings represent changes in the utilization of the inhibitor by the action of cortisone in the presence of large amounts of proteolytic enzymes produced by inflammatory states

## Metabolism of Porphyrins in Rheumatic Fever and Other Mesenchymal Diseases

DR ROBERT A ALDRICH Porphyrins are present in physiologically active forms in every living mammalian cell and are involved in the regulation of human physiologic processes Their elaboration is not so far as we know under the direct control of any of the glands of internal secretion

Every physiologically active porphyrin in the mammal contains an atom of iron However both type I and type III isomers of porphyrins are normally found in the free (uncomplexed) form in extremely small amounts in the excreta of mammals The physiologically active forms of protoporphyrin are hemoglobin myoglobin catalase the cytochromes and peroxidase In the plant kingdom chlorophyll also is a porphyrin but, instead of iron it contains an atom of magnesium

### *Porphyrinuria and porphyria*

By custom and common usage we reserve the term porphyrinuria for conditions in which there is an increased rate of excretion of porphyrin in the urine usually coproporphyrin and we reserve the term porphyria for certain clinically definable entities one feature of which is massive urinary excretion of uroporphyrin

The most common form of human porphyria is characterized by an outpouring of type III porphyrins both uroporphyrin and coproporphyrin in the excreta In this hepatic type of porphyria, the bone marrow is uninvolved and the porphyrins excreted apparently originate in the liver The nature of the metabolic error

that leads to this enormous excretion of porphyrins is still not com pletely understood There may be a metabolic block in the incorpo ration of iron into protoporphyrin to form heme or there may be another type of biochemical defect located elsewhere in the biosyn thetic sequence to protoporphyrin

### *Excretion of porphyrins in mesenchymal disease*

A significant coproporphyrinuria occurs in acute rheumatic fever rheumatoid arthritis polyarteritis nodosa and systemic lupus erythematosus More than 90% of the coproporphyrin excreted has the type III isomeric configuration

We have never found in acute rheumatic fever any evidence of a disturbance in the content of free coproporphyrin or free proto porphyrin in the erythrocytes but I would like to emphasize that most of our studies have been made during the first two or three weeks of the disease The normal content of these porphyrins in the erythro cytes early in rheumatic fever does not preclude the possibility that metabolism of porphyrin in the bone marrow is abnormal Most erythrocytes have a life span of 100 days or more, and those cells still in the bone marrow early in the disease may have an increased con tent of coproporphyrin or protoporphyrin in the free form Quanti tative assay of the amount of free porphyrins in bone marrow has been impossible to interpret

Our studies of the rate of excretion of coproporphyrins in the urine of patients with mesenchymal diseases have provided interest ing sidelights Despite reports to the contrary we have never seen administration of salicylates affect the rate of urinary excretion of coproporphyrins unless the salicylates were given in doses sufficient to produce toxic symptoms In one patient studied at the time of development of erythema marginatum a manifestation believed by some to indicate increased activity of rheumatic fever there seemed to be a definite increase in the urinary excretion of coproporphyrin

Figure 6 presents data obtained in a child whom we had the good fortune to study prior to development of manifestations of rheu matic fever Coproporphyrinuria was extreme prior to the onset of symptoms of acute rheumatic fever

### *Synthesis of heme*

In studies of the synthesis of heme we have obtained infor mation that may help to explain the significance of the coproporphy rinuria seen in mesenchymal disease The manner in which glycine and succinic acid condense to form alpha amino-beta ketoadipic acid and its subsequent conversion to delta aminolevulinic acid are now

quite well understood We know also that two molecules of delta aminolevulinic acid condense to form porphobilinogen Porphobilinogen or delta aminolevulinic acid can be converted to heme with ease by a variety of extracts from mammalian and plant cells Our knowledge of the way in which iron is incorporated into the protoporphyrin to form heme is meager

Using a system of lysed duck erythrocytes for studying the synthesis of heme Neve found that with the addition of glycine succinate and radioiron radioactive heme was formed Addition of

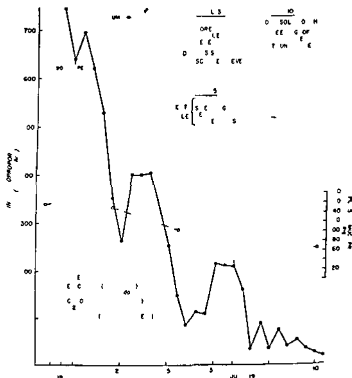


*Figure 6* Rate of excretion of coproporphyrin in the uri e and the concentration of mucop otein in the plasma of a child p or to and after the development of acute rheumatic fever During and immed ately after the attack of scarlet fe er which preceded the rheumat c fe er in this ch ld the rate of excretion of coproporphyrin in the urine was high but decreased to normal w th the development of manifestations of acute rheumatic fever Reprinted from Ped atrics *15* 557 (May) 1955

highly purified protoporphyrin did not result in any significant increase in the amount of radioheme formed indicating that protoporphyrin itself may not be in the direct pathway of heme synthesis However he found that when reduced uroporphyrin III was added to the same system there was a tremendous increase in the amount of radioactivity in the heme Thus reduced uroporphyrin III may lie in the direct pathway for synthesis of heme

The use of reduced protoporphyrin uroporphyrin I reduced uroporphyrin I or uroporphyrin III (not reduced) in this system produced practically no radioheme Reduced coproporphyrin III seemed to activate the system but not nearly as efficiently as did reduced uroporphyrin III Additional isotope dilution studies gave confirmatory evidence that reduced uroporphyrin III is in the direct pathway of synthesis of heme

These data suggest that reduced uroporphyrin III and possibly reduced coproporphyrin III might be important intermediates in the biosynthesis of heme Whether reduced coproporphyrin or reduced uroporphyrin actually accept iron I do not know Perhaps reduced uroporphyrin III combines with the iron atom and other enzymes decarboxylate the side chains of uroporphyrin to produce coproporphyrin Then if two carboxyl groups were changed to vinyl groups, iron protoporphyrin (i e heme) would result

We know that much synthesis of heme occurs in the cells of the liver and in porphyria the amount of synthesis in this site can be considerable It is certainly conceivable that coproporphyrinuria could result from a disorder in hepatic synthesis of heme in mesenchymal diseases It is also possible although as yet we have no evidence, that an abnormality in metabolism of porphyrins in mesenchymal diseases is localized in the bone marrow

## Discussion

CHAIRMAN GEORGE E CARTWRIGHT We have obtained evidence in our laboratory that protoporphyrin is an intermediate in the synthesis of heme Our experimental conditions are a little different from those of Neve and this probably explains the differences in the results

I would seriously doubt that the presence of coproporphyrinuria in itself indicates a serious error in metabolism of porphyrins Coproporphyrinuria is a very common finding as you know in a great many conditions

I was very much interested to hear that the content of free protoporphyrin and coproporphyrin in erythrocytes was found to be normal in acute rheumatic fever Our own studies are in agreement

with those of Aldrich for acute cases However we have found rather striking increases in the content of free protoporphyrin in the erythrocytes of children that have been sick for 60 days or more

DR R A ALDRICH It is certainly true that protoporphyrin will take up iron to form heme in vitro in the system used in the studies by Cartwright s group We have used the same phosphate buffer system and confirmed the e results

Whether the conditions we employed for synthesis of heme from reduced uroporphyrin III are more nearly physiologic than those employing the phosphate buffer remains to be settled It seems significant to us however that protoporphyrin is not converted to heme under these conditions I believe that the evidence favors a physiologic role for reduced uroporphyrin III and probably for reduced coproporphyrin III

## Anemia in Mesenchymal Disease

DR M EUGENE LAHEY Anemia accompanying mesenchymal diseases whether occurring in children or adults may be attributable to various causes such as concomitant deficiency of iron or loss of blood as occurs in the patient with anaphylactoid purpura This discussion however will not be concerned with anemia of such obvious cause but with the anemia of uncertain etiology that rather commonly complicates some of the mesenchymal diseases

What factors influence the development or the severity of anemia in these disorders have not been completely elucidated The following however appear to play a role The severity of the systemic reaction to the underlying disea e seems to be important, as indicated by the frequent occurrence of anemia during recrudescences in rheumatic fever and the restoration of hemoglobin values to normal during convalescence The duration of the disease would seem to be important In systemic lupus erythematosus for example patients not anemic at the outset often become so as the disease persists or progresses That the age of the patient is also important is suggested by the greater frequency of anemia in children with polyarteritis nodosa and rheumatoid arthritis than in adults with the same diseases Whether other factors, such as heredity previous nutritional status or emotional factors, play a role in the development of anemia is at present uncertain

## *Evidence for decreased rate of erythropoiesis*

The concept that anemia arises primarily as a result of de cr ased erythropoiesis rests largely on evidence from the study of patients with rheumatoid arthritis This evidence includes the in sidious onset of anemia reticulocytopenia the absence of cla sic signs of hemolysis, the diminution in number of erythroid elements and maturation arre t of normoblasts in the marrow In rheumatoid arthritis alterations in the metabolism of iron copper and porphyrins are strikingly similar to those in the anemia associated with chronic infections In the latter instance anemia is due primarily to decreased marrow function

## *Evidence for increased rate of hemolysis*

There are also observations that support the concept that ex ces ive hemolysis plays a role Convincing evidence has been presented that severe hemolytic anemia may be the first manifestation of systemic lupus erythematosus often antedating other manifestation of the di ea e by months or even years. Su h overt hemolytic anemia has occurred in 5 to 20% of adults and in almost one third of the small number of children studied The direct Coombs test is often positive even in the absence of obvious hemolysis This test, how ever is seldom positive in other me enchymal diseases

Hemolytic anemia has also been noted in a few patients with polyarteritis nodo a and in children with anaphylactoid purpura The signs of hemolysis have consi ted of sudden onset of anemia reticulocytosis normobla tic hyperplasia of the marrow aniso and poikilocytosis of mature erythrocytes and evidence of excessive ex cretion of products of the degradation of hemoglobin

The introduction of differential agglutination and i otopic tech niques has made it possible to inquire more thoroughly into the role of accelerated hemolysis in the anemia of various disease states It is now evident that though overt hemolysis may be uncommon minor degrees of hemolysis are not infrequent in at lea t some types of me enchymal disease In rheumatic fever the survival of normal cells has been shown to be reduced by 15 to 20% Similarly in rheumatoid arthritis, the life span of the erythrocytes may aver age only 70 to 100 days instead of the usual 110 to 120 days These obs rvations have been made after the admini tration of normal cells to patients with these disorders When cells from the patients how ever are given to normal recipients, the survival time is normal in dicating that the hemolysis is a result of extracorpuscular rather than intrinsic or intracorpuscular defects

## *Possible causes of decreased rate of erythropoiesis and increased rate of hemolysis*

Certain hypotheses can be offered to explain the occurrence of either decreased function of the marrow or accelerated destruction of erythrocytes as a cause of anemia in mesenchymal disease Some evidence suggests that the primary cause of lessened erythropoiesis lies in defective synthesis of hemoglobin The normal or increased content of protoporphyrin in the erythrocytes suggests that early steps in the synthesis of hemoglobin are not affected It may be that the defect exists at the point where iron is incorporated into the porphyrin molecule to form heme or that the fault lies in the fabrication of globin Since the metabolism of other proteins is seriously affected in these disorders it is not unreasonable to suspect that the erythrocyte is affected simply because there are other more important uses for iron protein and other constituents during the active phase of the disease

It has been postulated that a variety of stimuli—chemical bacterial viral et cetera—may provoke changes in the erythrocyte so that it becomes antigenic with ensuing autoimmune hemolytic disease Just as some individuals form antibodies to erythrocytes with remarkable facility after transfusions of blood some may also form antibodies with the same facility in response to whatever stimulus provokes the mesenchymal disorders Such an altered immunologic reaction may account for the hemolytic anemia and the positive Coombs test noted especially in patients with systemic lupus erythematosus Antibodies may have an adverse effect not only on mature erythrocytes but also on reticulocytes and even on their nucleated precursors in the marrow

## *Considerations regarding attempts at correction of the anemia*

The anemia of mesenchymal diseases is usually mild and the welfare of the patient does not seem to be jeopardized by the degree of anemia usually encountered nor does correction of the anemia hasten recovery Vigorous therapy directed against the anemia per se is therefore seldom warranted especially when we consider the greater frequency of reactions to transfusions in these patients In occasional instances of brisk hemolysis such as occur not infrequently in systemic lupus erythematosus, transfusions of blood may be life saving

Anemia usually subsides in those patients successfully treated with adrenocorticotropin cortisone or a related substance It is probable that improvement comes from the effect of these agents on

the underlying disease In patients with systemic lupus erythematosus or rheumatoid arthritis complicated by hemolytic anemia or pancytopenia splenectomy has occasionally been performed but usually with only transient hematologic improvement The scattered reports that administration of iron is of value in alleviating the anemia of rheumatoid arthritis are probably based on improvement coinciding with spontaneous recovery from the underlying disease or to correction of a coexisting deficiency of iron Many other therapeutic agents have been tried for the anemia of mesenchymal diseases but their value has not been substantiated and definite risk may attend their use

# Biosynthesis of Adrenal Steroids

DR LEO T SAMUELS The more important hormones of the adrenal gland are 17 hydroxycorticosterone (cortisol or compound F) corticosterone aldosterone 11 beta hydroxyandrostenedione androstenedione and probably estrone All have a four ring tructure the cyclopentenophenanthrene nucleus

### *Synthesis of cholesterol*

The importance of cholesterol in the formation of the adrenal steroids was recognized first by observation of the decrease in content of cholesterol in the adrenal gland after stress The isolation of radioactive adrenal steroids after perfusion of radioactive cholesterol through adrenal glands demonstrated that cholesterol could be a pre cursor of the adrenal hormones

Cholesterol can be synthesized also from acetate in the adrenal gland and it seemed possible that the acetate incorporated in adrenal hormones might have been incorporated in cholesterol as an inter mediate

As indicated in figures 7 and 8 the steps in the synthesis of cholesterol from acetate appear to involve the following intermediates acetylco A acetoacetate and beta hydroxy beta methylglutaric acid The la t sub tance if labeled with radioactive i otope and introduced into a homogenate of liver or adrenal gland will give rise to radioactive squalene and cholesterol Oxygen is necessary in the reaction although the overall re ult is a reduction Triphosphopyridine nucleo tide in reduced form is also necessary

### *Synthesis of various hormones of the adrenal cortex*

Using the supernatant fluid from a homogenate of the adrenal glands under controlled conditions of pH and in the presence of triphosphopyridine nucleotide, Guerin has found that cholesterol is converted rapidly to pregnen 3 beta ol 20 one a compound closely related to the adrenal hormones and found in both the adrenal glands and te tes

$$12\ CH_3CO\ CoA \rightarrow 6\ CH_3COCH_2COOH\ (6H_2O) + 6\ CH_3CO\ CoA \rightarrow$$

$$6\ HOOCCH_2\underset{OH}{\overset{CH_3}{C}}CH_2COOH\ (+6CO_2) \rightarrow 6\ CH_3\underset{OH}{\overset{CH_3}{C}}CH_2COOH\ (+6CO_2) \rightarrow$$

$$6\ CH_3\overset{CH_3}{C}{:}CHCOOH\ (+6H_2O) \rightarrow 2\ CH_3\overset{CH_3}{C}{:}CHCH_2CH_2\overset{CH_3}{C}{:}CHCH_2CH_2\overset{CH_3}{C}{:}CHCOOH \rightarrow$$

$$CH_3\overset{CH_3}{C}{:}CHCH_2CH_2\overset{CH_3}{C}{:}CHCH_2CH_2\overset{CH_3}{C}{:}CH_2CH_2CH{:}\overset{CH_3}{C}CH_2CH_2CH{:}\overset{CH_3}{C}CH_2CH_2CH{:}\overset{CH_3}{C}CH_3$$

*Figure 7* St ps in biosy thes s of squalene ($C_{30}H_{50}$)

SQUALENE LANOSTEROL CHOLESTEROL

*Figure 8* B osy thes s of cholest l f om sq al ne

*F g e 9* St ps l d l osy thes f l l h m es f m L p gne l

Heard has been able to synthesize the adrenal steroids from acetate in a cell free system Previously such synthesis had been done with systems containing organized cells Utilizing radioactive labeled acetate in this cell free system cortisol with high radioactivity was produced, but no radioactive cholesterol as though under these circumstances *there had been no conversion to cholesterol, and all the* acetate had gone by some other route into the adrenal hormones These and other findings suggest that cholesterol may not be an indispensible intermediate in the formation of adrenal hormones

Figure 9 indicates the general reactions that are probably involved in the synthesis of progesterone 17 hydroxyprogesterone desoxycorticosterone 17 hydroxycorticosterone (cortisol) corticosterone androstenedione and 11 beta hydroxyandrostenedione Evidence has been presented by Hechter that these reactions must occur in sequence Perfusion of the adrenal gland with 17 hydroxyprogesterone resulted in production of substance S which includes the 21 hydroxy group and of cortisol but not of corticosterone When desoxycorticosterone was perfused corticosterone but not cortisol was obtained Similar *results suggesting that these reactions must take place in sequence* were obtained from our studies in vitro It has been shown more recently that this sequence is not obligatory but under the conditions usually found in normal cells hydroxylation ordinarily does occur in this sequential fashion

It seems that the presence of tripho phopyridine nucleotide in the reduced form is necessary whenever replacement of hydrogen with hydroxyl must take place on a —CH — or >CH— group If the reaction is a simple dehydrogenation with the conversion of an hydroxyl group to a ketone or the formation of a double bond then oxidized diphosphopyridine nucleotide is needed on a $CH_2$ group These are the major coenzymes in steroid biosynthesis

Aberrations in the various enzyme systems may result in formation of androgenic or estrogenic tumors or of tumors that might simply produce excess amounts of corticosterone cortisol or aldosterone By knowing these steps in biosynthesis we can look for compounds that might compete with the usual substrates of abnormally functioning enzymes, and thus stop the production of undesirable hormones New compounds have been discovered that do seem to compete in this fashion to some extent

## Pro- and Antiphlogistic Steroids

Dr Thomas F Dougherty The steroid hormones may be divided into those which tend to diminish the degree of inflammatory

response called antiphlogistic and those which tend to increase the inflammatory response called prophlogistic

## *Theoretic considerations*

Selye has suggested that there is a balance between the amounts of anti and prophlogistic steroid hormones secreted by the adrenal cortex and that the defect responsible for the production of mesenchymal diseases may be an alteration in adrenal cortical secretion He believes that an increased output of prophlogistic hormones may counterbalance the anti inflammatory effects of such compounds as cortisol (hydrocortisone)

By this hypothesis desoxycorticosterone is the prototype of the prophlogistic hormones The naturally secreted prophlogistic hormone is aldosterone which is approximately as prophlogistically potent as desoxycorticosterone Growth hormon may be the trophic hormone for secretion of aldosterone

Our studies indicate that desoxycorticosterone markedly reduced the resistance to anaphylactic shock and to local inflammation induced by allergic reactions When desoxycorticosterone is given *concurrently* the anti inflammatory and antianaphylactic capacities of cortisone are reduced However we have no definite evidence that the adrenal cortex in mesenchymal diseases is functionally altered in such a way that aldosterone predominates in the secretion of the gland and we have no convincing evidence that growth hormone is the *trophic hormone for aldosterone*

## *Technique of study*

Subcutaneous tissue is spread on a slide so that it is practically one cell thick and it is then possible to count the number of cells in an area 1 cm on a side and to calculate the number of cells in 1 $cm^3$ of tissue The method of counting is analogous to that of the ordinary blood count.

Figure 10 demonstrates the appearance of a spread of tissue from an area of inflammation produced by injection of a standard amount of gelatin a substance evoking a mild inflammation of consistent appearance Also shown is the appearance of tissue from a similarly treated area of an animal that had received cortisol in addition to the gelatin The anti inflammatory effect of cortisol is apparent

By this technique it was shown that cortisol is a more potent anti inflammatory steroid than cortisone Study of analogs of the potent hormones makes it possible to ascertain the structural relationship that determines the degree of potency provided by an anti inflammatory hormone

We find that the unsaturation of the A ring is absolutely essential for anti inflammatory effect The presence of the 17 hydroxy group is also essential and the presence of an hydroxy or oxygen

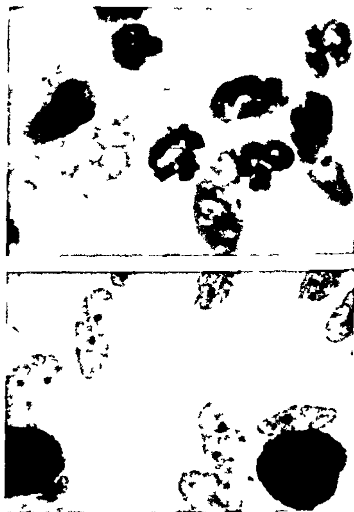


*Figure 10* Appearance of a spread of tissue prepared from an area of inflammation produced by injection of gelatin (upper) and of tissue from a similar area of an animal that received hydrocortisone in addition to the gelatin (lower) The absence of polymorphonuclear cells and of other evidences of inflammation is apparent in the tissue from the animal that received hydrocortisone

group at the 11 position produces marked potentiation of the anti inflammatory effect We hope to study the whole series of hormones that apparently occur naturally in the adrenal cortex

Some years ago we established the fact that the anti inflammatory effect of cortisol is exerted at the site of its presence and that it is not related to or dependent upon circulation through the liver Thus cortisol itself rather than one of its conversion products appears to give the anti inflammatory effect

Radioactive (4 $C^{14}$) cortisol was given intravenously to adrenal ectomized animals at the same time that an inflammation was produced by injection of gelatin into one flank the opposite flank acting as control noninflamed tissue The concentration of hormone in noninflamed tissue paralleled the pattern of that in inflamed tissue although the inflamed tissue accumulated more of the radioactive hormone

In studies of the rate of conversion of cortisol to glucuronide by the liver we demonstrated that the concentrations of conjugated and nonconjugated hormone are approximately the same in the blood as in the area of inflammation Because conjugation of cortisol occurs only in the liver the similarity of concentrations in blood and in inflamed tissue indicates ready transit of the hormone between these two areas

In making radioautographs after subcutaneous administration of radioactive cortisol we have learned that the radioactive substance tends to localize intracellularly in the fibrobla t and fat cell but that the duration of its presence in these cells is rather short compared with the prolonged anti inflammatory effect it produces Therefore we suspect that it is an alteration at the fibroblastic cell level which the hormone produces in the tissue rather than its continued presence that is essential for anti inflammatory activity

## Discussion

Dr Dorfman Schiller and I have been trying for a long time to study directly the effect of cortisol on synthesis of polysaccharides When we pretreated animals with cortisol there was a marked inhibition of synthesis of both hyaluronic acid and chondroitin sulfate *in the skin* *Maximum inhibition did not occur for about five days*

Dr Dougherty Layton also found a delay between the admin*istration of cortisone and appearance of inhibition in formation of* ground substance

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The hormone labeled isotopically disappears from the organism within about 48 hours It cannot be identified in a localized site within a much shorter period of time

*It is likely that the many actions of cortisol are the result of* its effects on many different systems It may not have to be present in excess when its activity is demonstrated by any physiologic test It does not surprise me that *inhibition of synthesis of polysaccharides* does not occur for some time after the cortisol is administered

DR DAVID GLICK Would you comment on the claim that cortisol causes destruction or disruption of the mast cells?

DR DOUGHERTY I do not believe we can say that there is any specific effect of cortisol in disruption of mast cells

# Quantitative Histochemical Studies of the Adrenal Gland in Various Physiologic States

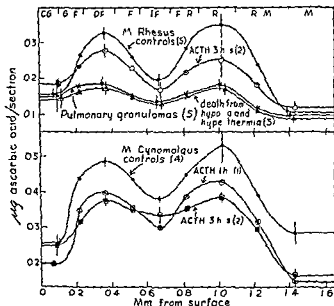
DR DAVID GLICK We chose the quantitative histochemical approach in an attempt to obtain information about the localization in the adrenal gland of some of the important biochemical processes important in its functioning We worked out a method of killing animals suddenly with what we believed would be minimal stress Within 30 seconds after an animal had been peacefully sitting in his cage with no anticipation that anything would happen his adrenals were frozen in dry ice The adrenals *were then not per*mitted to thaw until the final chemical analysis or histologic study was made

A cylinder of tissue was drilled from the frozen adrenal and mounted on a rotary microtome Sections were taken through all the zones of the cortex and through the medulla Individual sections were used for chemical analysis and adjacent ones for histologic identification so that a fairly good degree of correlation could be achieved between histologic and chemical findings All of the analyses were performed by colorimetric or spectrophotometric methods some*times on volumes of fluid as small as 0 005 ml*

### *Studies of ascorbic acid and cholesterol in the adrenal cortex*

Ascorbic acid is lost suddenly from the adrenal gland when the organ is stimulated either hormonally or by stress Represented

graphically in figure 11 are the results of our studies to determine in what zones of the adrenal cortex thi lo s occurs and the actual amounts of a corbic acid lo t as the result of a given treatment When monkeys were given a single ubcutaneous injection of adreno corticotropin three hours before sacrifice a decrease in total con centration of ascorbic acid was observed If the animals were stressed by a combination of hypoxia and hypothermia there was an even more drastic decrease When rats were put to death by ether or were




*Figu e 11* Th concentr t on of ascorl c a i l in ar o s porti ns l the drenal gland of the u (upper) and cynomolgus (low r) monkeys under con i tions of min mal stress (highest curve) and afte adm i i at on of adrenocort cotropin or production of t e.s in a i is ways The l tter along tl top of th fig re in l te the hi tolog z ne of the adrenal glan l C-c psul G-glomerulo a OF-oute fasc ulata, IF n fasc ulata R ret ula is, M m dulla Reprinte l from Endocrinol gy 51 6 6 (June) 1951

given an injection of adrenocorticotropin one hour before sacrifice the same general effect was obtained If they were merely held in the hand causing them to truggle a maximum loss of ascorbic acid occurred within two minutes

Lo s of cholesterol also occurs when the a lrenal glands are timu lated hormonally or when the animal is subjected to environmental

stress In the unstressed cynomolgus monkey we found that practically all the cholesterol in the adrenal was in the form of ester It was concentrated in the outer fascicular zone and to a lesser degree in the reticular area Three hours after injection of adrenocorticotropin the concentration was markedly decreased On the other hand injection of cortisone produced no effect on the content of cholesterol in any zone

### *Studies of enzymes that deconjugate hormones*

Beta glucuronidase an enzyme present in appreciable amounts in the adrenal is known to liberate free hormone from the conjugated form We calculated the content of beta glucuronidase in the various zones of the adrenal cortex of the rhesus monkey by determining the rate of liberation of phenolphthalein A peak concentration of beta glucuronida e was found in the reticular zone in both the untreated animal and the animal treated with adrenocorticotropin with no real difference in concentration between the two

In this zone the content of protein nitrogen was also elevated We believe that this determination represents a better index of concentration of active metabolizing enzyme than does dry weight or volume of tissue because especially in the adrenal so large a proportion of the tissue is lipid substance

We found remarkable differences in concentrations of beta glucuronidase in the adrenal among animals of different pecies For example in bovine adrenal there is a definite high concentration in the glomerular zone and less activity in other zones

After hypophysectomy the adrenals of rats undergo atrophy and weigh only one half as much as in control animals Histologically the relative distribution of beta glucuronidase in the adrenal is unchanged The highest concentration is still in the reticular zone but the magnitude is increased remarkably

We speculated that this might mean increased active free hormone liberated from conjugate circulating in the blood and picked up by the adrenal While the need for adrenal hormone continues because of atrophy the organ cannot respond with production of hormone Since hormones are conjugated to sulfate as well as to glucuronic acid deconjugation by phenolsulfatase might also be expected to occur in the adrenal

The concentration of phenolsulfatase was found to be greatest in the fascicular region and no change was produced by prior treatment with desoxycorticosterone acetate adrenocorticotropin or cortisone in the doses we employed Similarly chilling or formation of a sterile abscess by injection of turpentine had no effect on the concentration of phenolsulfatase The activity remained remarkably

constant in spite of very drastic environmental changes Six days after hypophysectomy at a time when a great deal of atrophy of the adrenal had already occurred instead of an increased concentration as with beta glucuronidase there was actually a decrease in concentration of phenolsulfatase I do not know why there should be this difference in the effect of hypophysectomy on the level of the two enzymes both of which deconjugate hormones

### *Studies of cholinesterase succinic dehydrogenase and coenzyme A*

Cholinesterase is concentrated in the medulla of the adrenal gland of the rat there is almost none in the cortex as might be expected since the neural elements in the gland are concentrated in the medulla After administration of a variety of hormones and production of stress by various means no effect on the concentration of cholinesterase was observed The atrophy of the adrenal resulting from hypophysectomy also effected no change in concentration of cholinesterase The concentration of succinic dehydrogenase an enzyme involved in the Krebs cycle was also unaffected by administration of adrenocorticotropin or cortisone

Deficiency of pantothenic acid results in decreased rate of liberation of adrenal hormones Coenzyme A a derivative of pantothenic acid is essential for the production of cholesterol from acetate in the adrenal We found that the peak of distribution of coenzyme A is in the reticular zone After treatment with adrenocorticotropin or desoxycorticosterone there was little change There was no change at all six days after hypophysectomy as compared to the sham operated control

It may be that for certain important enzymes in tissues a remarkably constant concentration is maintained even in spite of pharmacologic influences Possibly these enzymes are so essential for the function of the tissues that an excess is always supplied

## Response of the Pituitary-Adrenal System to Psychic Stimuli

Dr Eugene L. Bliss The work that I am about to report was undertaken in an attempt to learn whether psychologic disturbances in man were associated with alterations in adrenocortical function

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[illegible] the degree to which hormonal secretion, as reflected in [illegible] 17-hydroxycorticosteroid in the plasma might [illegible] normal levels.

[illegible] to measure accurately the concentration [illegible] in the plasma, the estimate of [illegible]. No attempt was made to distinguish [illegible] emotion, such as fear, rage [illegible] of emotional disturbance exhibited [illegible] myself.

[illegible] were graded as either [illegible] disturbed. At other times, emotional [illegible] from 0 to 3, in which 0 represented [illegible]. All estimates of emo[illegible] of chemical results.

### Studies of psychotic patients

[illegible] patients was studied [illegible] the psychiatric ward. Seven [illegible] emotionally disturbed were found [illegible] 17-hydroxycorticosteroid in the [illegible] value of 13 μg/100 ml in [illegible].

[illegible] the hebephrenic variety [illegible] and whose manifest [illegible] low, had concentra[illegible] within normal limits. [illegible] with chronic schizo[illegible] in the blood because [illegible] hallucinations, bizarre [illegible] in schizo[illegible] concentration of 17-hy[illegible].

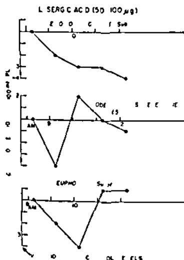
### [illegible] normal subjects

[illegible] their [illegible] 17-hydroxy[illegible].

[illegible] the blood [illegible]. Under these [illegible] and the concentration of 17-hy[illegible] in the blood rose during the period [illegible].

From other studies it has become evident that the increases in concentration of 17 hydroxycorticosteroids in the pla ma during emo tional stress are relatively modest in compari on with the effect of other stressful procedures Most potent of the procedures tudied is administration intravenously of adrenocorticotropin

The trends in our studies were remarkably consistent Increa es in the concentrations of 17 *hydroxycorticosteroids in the blood aver* aged 5 to 7 µg/100 ml with emotional stress All values were within the known physiologic limits even in the face of the mo t disabling



*Figu e 12* The effect of ad mini trat n f *d* lyser i aci l to normal sul ject In fi e in d d l who pe len d l t tl r no emot o al ı set th conc ntrat on of l hyd y cortico teroids in the pla m ere within normal lim ts, d creas n n tl e normal d urnal p ttern In f e other sul je t who b c me q t di turb t th o t at ns of the ster o d we e le ated d ring the p d of ma mum emotional d tu b Four ul jects d lop d a e ph i response wh h wa assoc ted with in c e in e trati n of 1 hydro y ort oste oid in th blo d Reprinted from I ych somat c Me licine *18* 65 19 6

and catastrophic panic Because of the consistency of the re ults in a variety of individuals studied under diverse types of emotional stress it seems likely that acute tates of emotional di turbance and heightened anxiety are routinely although perhaps not invarial ly associated with small but significant increases in the concentrations of 17 hydroxycorticosteroids in the blood Since there is also an increase in the rate of excretion of the 17 hydroxycorticosteroids in the urine it seems likely that emotional stress is associated with a modest in crease in adrenocortical activity

### *Influence of central nervous system*

It seems probable that the central nervous sy tem influences the anterior pituitary gland via hypothalamic centers We have tentative indirect evidence of the relation hip between the central nervous system and the endocrine glands When patients receive electroshock

therapy there is an increase with the grand mal seizure of the concentration of 17 hydroxycorticosteroids in the plasma When these seizures are blocked by suitable anticonvulsive agents so that the *muscular movements are inhibited except for a slight twitching* of the eyelids the concentration of 17 hydroxycorticosteroids increases nevertheless

In decerebrate human subjects the concentration of 17 hydroxy corticosteroids in the plasma is consistently greater than in normal subjects

It seems that electrochemical and psychologic processes arising in the cerebral cortex have an important effect upon the activity of the anterior pituitary gland which in turn causes the adrenal cortex to modify its rate of secretion Under the impact of acute emotional stress the concentration of 17 hydroxycorticosteroids in the blood will increase The degree of elevation is usually modest and remains within physiologic limits Cortical discharge induced by electroshock will also produce elevations in these concentrations even when peripheral *convulsive activity is blocked*

## Pituitary-Adrenal Function in Mesenchymal Diseases

### *Evidence of endocrine disturbance in mesenchymal diseases*

DR ROBERT S ELY The first clinical trials with cortisone were performed in patients with rheumatoid arthritis because clinical experience had suggested the possibility that adrenocortical function has an influence on the course of the disease Subsequently reports of the beneficial effects of cortisone and adrenocorticotropin in other mesenchymal diseases strongly invited the belief that an altered pituitary adrenal function was present

A relationship between mesenchymal diseases and adrenal function was also suggested by studies of certain acute phase reactants particularly the nonspecific hyaluronidase inhibitor The concentration of this inhibitor in the plasma is lower than normal in adrenalectomized or hypophysectomized animals, and administration of adrenocorticotropin or cortisone produces a rise Inhibitor concentration is low also in patients with rheumatic fever except during the early acute phase of the disease when it is elevated as it is in other acute illnesses

Animal experimentation has shown a definite influence exerted by hormones on the function of connective tissue The mineralocorticoids, or phlogistic corticoids enhance reactivity of connective tis ue whereas the glucocorticoids tend to act in an opposite manner De pite this it is known that administration of either type of hormone can under experimental conditions induce in animals lesions similar to those of mesenchymal diseases

### *17 hydroxycorticosteroids in acute rheumatic fever*

We have been interested for some time in evaluating pituitary adrenal function in children with rheumatic fever and related diseases Certain abnormalities in pituitary adrenal function seem to be present although their significance lies beyond interpretation at the present time In children with early acute rheumatic fever — that is during the first week of rheumatic activity — the concentration of 17 hydroxycorticosteroids in plasma is approximately twice as great as those of control children It should be mentioned here that patients with other acute illnesses commonly have concentrations higher than those seen in patients acutely ill with rheumatic fever In patients who have had active rheumatic fever for at least two weeks the concentration of 17 hydroxycorticosteroids is consistently low In patients with inactive rheumatic fever the concentration is also low

### *Adrenal insufficiency*

This low concentration of 17 hydroxycorticosteroids in the pla ma is interpreted by us as indicating the presence of adrenal insufficiency The concentration does not appear to be low because of an increased rate of utilization by tissue since the rate of disappearance of administered steroid actually is lower than normal

If the adrenocortical hypofunction were primary rather than secondary to insufficient stimulation by the pituitary we would expect increased secretion of adrenocorticotropin by the pituitary to be associated with the decreased secretion of steroid by the adrenal gland In patients with rheumatic fever except during the early acute phase the concentration of 17 hydroxycorticosteroids in the plasma is lower than normal de pite an elevated concentration of adrenocorticotropin Since the rate of disappearance of these steroids from the circulation is not increased these data are interpreted as indicating that the patient with rheumatic fever has primary adrenal in ufficiency

That adrenal insufficiency is relative rather than absolute is indicated by the significant increase in concentration of 17 hydroxycorticosteroids in the pla ma after administration of a large test dose of adrenocorticotropin The nature of the in ufficiency remains somewhat obscure Dimini hed concentration of 17 hydroxycorticosteroids in the plasma is not reflected in decreased rates of urinary excretion

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The occurrence of a segmental adrenal insufficiency in certain conditions is known In congenital adrenal hyperplasia the patient produces only small amounts of 17 hydroxycorticosteroids, although he consistently produces an excessive quantity of certain other ter oids A specific deficiency of one of the many enzymes involved in steroidogenesis appears responsible for the abnormal metabolism in this disease Study of other steroids eemed desirable in children with mesenchymal diseases and it was found that, in active and inactive rheumatic fever and in rheumatoid arthritis, the mean concentration of corticosterone in the plasma is significantly higher than in control subjects

Responsiveness of the adrenal gland is clearly dependent on nor mal metabolism of the circulating steroids The factors involved in steroid metabolism other than conjugation and excretion are not well known However one approach to the problem consi ts of determin ing the half life of administered steroid We studied only the rate of removal of steroid in the free form and have no data bearing on the rate of conjugation or of excretion The mean half life of cortisol or corticosterone in active rheumatic fever and rheumatoid arthritis was significantly greater than in control children of similar age

## *Studies of families of patients with rheumatic fever*

In considering factors that determine the reaction of the ho t in the production of mesenchymal diseases one postulate is that rheu matic susceptibility is genetically determined If a hereditary physio logic or biochemical factor were responsible for this rheumatic susceptibility it might be demonstrable in families of patients Be cause of the consi tent finding of low concentrations of 17 hydroxy corticosteroids in the plasma of subjects with rheumatic fever we undertook a study of their families

In table 5 the concentrations of 17 hydroxycorticosteroids and of corticosterone in plasma of parents and siblings of patients with rheumatic fever are shown The concentration of 17 hydroxycorti *costeroids is significantly decreased in parents and* siblings, whether these individuals had positive histories of rheumatic fever or not On the other hand the concentration of corticosterone in the plasma of the siblings is greater than normal paralleling the increased con centration seen in children with rheumatic fever and other mesen chymal diseases The parents, however do not show a significant alteration

The significance of these evidences of abnormal adrenal function and abnormal metabolism of corticosteroids in the pathogenesis of mesenchymal disease is not clear

Table 5

*Concentration of 17 hydroxycorticosteroids and of corticosterone in the plasma of members of families of patients with rheumatic fever*

| Group | Number of Subjects | Mean Plasma Concentration (µg/100ml) | Standard Error of Mean |
|---|---|---|---|
| | | 17 OHCS | |
| Control | 40 | 12.0 | ±1.29 |
| Siblings | | | |
| +History R.F. | 6[illegible] | 7.5 | ±0.68 |
| −History R.F. | 143 | 6.9 | ±0.16 |
| Parents | | | |
| +History R.F. | 16 | 7.7 | ±1.53 |
| −History R.F. | 6 | | ±0.63 |
| | | Corticosterone | |
| Controls | | | |
| 5-17 years | 31 | 3.[illegible] | ±0.30 |
| Adults | [illegible]1 | 3.0 | ±0.[illegible]1 |
| Siblings | | | |
| +History R.F. | 29 | 6.3 | ±1.[illegible] |
| −History R.F. | 6 | 6.6 | ±0.91 |
| Parents | | | |
| +History R.F. | 7 | [illegible]5 | ±0.49 |
| −History R.F. | 3[illegible] | [illegible]4 | ±0.5 |

In patients with rheumatic fever the relative adrenal insufficiency consisting of a low concentration of 17 hydroxycorticosteroids and elevated concentrations of corticosterone and adrenocorticotropin in the blood raises the vital question does this pattern of adrenal function precede the first attack of rheumatic fever or is it a result of the attack? Similar findings in the plasma of patients whose rheumatic fever has been inactive for many months suggests that it precedes the rheumatic attack. Even more important the presence of these same findings in otherwise normal siblings of patients with rheumatic fever is strongly suggestive that altered adrenal function antedates the first attack of rheumatic fever

# Juvenile Rheumatoid Arthritis

Dr Thomas A Good This report deals with data concerning 73 cases of juvenile rheumatoid arthritis studied during the last 10 years

Still s triad of findings for the diagnosis of juvenile rheumatoid arthritis consisted of arthritis lymphadenopathy and splenomegaly Because of the variability of manifestations of juvenile rheumatoid arthritis there seems to be no good reason to separate Still s disease from other forms

In young children other manifestations of Still s disease frequently precede the development of noticeable arthritis and sometimes deforming arthritis may not be manifest at all before a spontaneous remission occurs Therefore early recognition of signs makes possible diagnosis for prognostic purposes and better direction of management

### *Clinical manifestations*

In the small child illustrated in figure 13 the presumptive diagnosis may be made by noticing the characteristic posture and the facies he presents He desires to be left alone and remains very still holding his hands and grimacing in a worried fashion In bed he shows a marked lack of spontaneous movement usually lying on his back with all extremities flexed These are the signs of arthralgia and myalgia in the small child

A spiking temperature curve in a patient who acts considerably less ill than would be suspected from the degree of fever sugge ts the diagnosis of juvenile rheumatoid arthritis however progression of the disease may lead to severe toxicity and emaciation requiring intensive supportive management, (figure 14) In the older child the primary manifestation is likely to be arthritis of multiple joints with swelling contracture and deformity as in the adult

Rarely seen in any other disease and therefore a main diagnostic criterion is the spindling of the fingers This may be recognized early in the disease even in an infant (figure 15)



*Figure 13* Worried fa ial appearance and cha acter t c po ture juvenile rheumatoid a th t s.

Of importance in the diagnosis is the occurrence of a ra h that appears to l e as diagnostic for juvenile rheumatoid arthritis as the rash of measles is for that disease The primary lesions are small discrete salmon pink macules with pale centers Coalescence of the macules frequently makes the rash appear blotchy This ra h is evanescent usually appearing suddenly with fever and disappearing rapidly

Tal le 6 shows the frequency of the various clinical manifestations of juvenile rheumatoid arthritis in 75 patients between 6 months an l 15 years of age

Confu ion with rheumatic fever often arose when signs of carditis were elicited Systolic murmurs of gra le II inten ity were heard not infrequently during the toxic pha es of the disease Tachycardia and non pecific electrocardiographic changes con i ting mostly of inver ion of T 2 and T 3 and light prolongation of the I R and QT intervals were seen No definite heart block or evidence of residual carditis has been seen

## *Laboratory findings*

Laboratory findings were non pecific for juvenile rheumatoid arthritis The sedimentation rate was elevated elevation of the globulin caused reversal of the A/G ratio marked leukocytosis frequently



*Figure 14* Cachectic appearance in Still s di ease

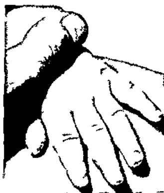


*Figure 15* Spindling of fingers recognizable early in juvenile rheumatoid arthritis detectable even in the pudgy hands of young children

occurred and anemia often was severe The most common roentgenographic joint abnormality was periarticular osteoporosis Mild albuminuria occasionally spurred the hunt for indolent urinary tract infection Bacteriologic serologic and kin tests helped to rule out arthritis of infectious origin Except for increased plasma globulin studies of liver function failed to disclose abnormalities The concentration of mucoproteins in the serum ranged widely with the mean

## Table 6

*Clinical manifestations in juvenile rheumatoid arthritis*

| S g o symptom | F q cy I oc |
|---|---|
| A thralgia | 9,% |
| Arthritis of multiple jo nts | 95% |
| Fever | 9 % |
| Myalgia | 65% |
| Spindl ng of finge | 5 % |
| Lyhadenopathy | 51% |
| Skin ra h | 48% |
| H pato pl nom galy | 40% |

## Table 7

*Features important in the differential diagnosis of juvenile rheumatoid arthritis and rheumatic fever*

| Ma f t t | J I Rh m t d A th t | Rh m t c F |
|---|---|---|
| Arthritis | I i dolent<br>Seld m migrat ry<br>Tend t in olve small jo ts<br>Def rming | F a sce t<br>Regula ly m g at ry<br>Tend t I la g joint<br>Ne r def rming |
| Fe r | Sept c temp r tu e curve comm n | Sept t mperature curv t mely ra e |
| Splen megaly an I g ner l z d a l opatl y | Comm n | Rare |
| Sk ra h | F escent, blot hy eryll emat m ules w th wh t t rs | Eryth ma m g natum ryth ma m lt f m ryth ma I m |
| Respo se to sal cyl tes | Poor | D mat c |
| Val ular h art d sease | No | Comm n |
| Ir I g t fect n by str ptoroc n | L u I | R gular |

value more than three times normal Titers of antistreptolysin O in the blood were helpful in differentiating juvenile rheumatoid arthritis from rheumatic fever Of 39 patients only 6 had titers greater than 250 Todd units The three major L E tests were invariably negative (186 tests on 58 patients)

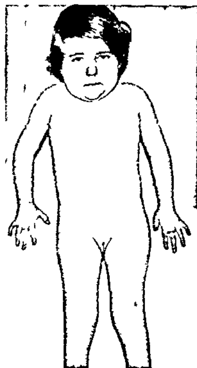


*Figure 16* Dramatic response to steroid therapy of patient in figure 14 Note Cushinoid side effects

## *Clinical findings in relation to age*

Of our patients manifesting lymphadenopathy hepatosplenomegaly spiking fever and rash 86% were less than six years of a e On the other hand 87% of patients with spiking fever typical rash and arthritis but without lymphatic hypertrophy had the onset of their manifestations after the age of six years Patients who had primarily arthritis of the more indolent nature were u ually more than six years of age The marked hypertrophy of the lymphatic tissue in these children has the same age distribution as that seen in chronic diseases known to be caused by infectious agents and this is interpreted as indirect evidence supporting the possibility that juvenile rheumatoid arthritis has an infectious etiology

Since etiology and pathogenesis are obscure no rationale for therapy exists Treatment is aimed at the prevention of joint disability This should be gentle especially when given with hormone therapy Rest in bed does not seem to modify the course of the disease and may actually be harmful in contributing to the production of contractures Salicylates appear to be useful in relieving pain and stiffness Hormones appear to suppress the disease but do not induce lasting remission Dramatic results are often experienced with hormone therapy as seen in figure 16 The suppression of the disease produced by adrenal corticoid therapy appears to be related to the levels of 17 hydroxycorticosteroids in the blood (figure 17) However since the disease is characterized by long period of activity complications of hormone therapy are much more troublesome than in rheumatic fever Treatment therefore is directed primarily toward relieving joint symptoms to facilitate physiotherapy In cases of inanition hormone therapy is helpful because of its stimulating effect upon the appetite

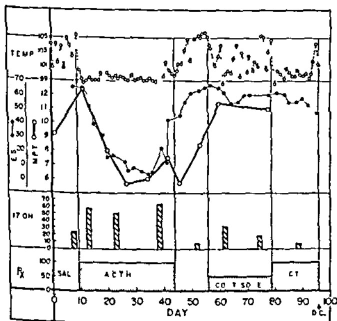



*Figure 17* Relationship of plasma 17 hydroxycorticosteroid levels to the suppressive effect of adrenal cortical hormone therapy on fever erythrocyte sedimentation rate [illegible]

Because of apparent hypersusceptibility to infection early specific antibiotic therapy for intercurrent infection is indicated No deaths resulted from superimposed infection and amyloidosis was not seen in the group studied With individualized management the prognosis for life in juvenile rheumatoid arthritis is excellent and many patients recover without severe sequelae However in some the disease is characterized by repeated attacks and progressive joint disability

## Systemic Lupus Erythematosus

### *Studies of splenic aspirates*

DR MALCOLM M HARGRAVES For our hematologic studies we have used smears of material obtained by splenic puncture The preparations are made on chemically clean slides from the material in the needle itself Cells developing in the ground substance of the spleen can be rolled out on the slide and are not as disrupted as those from aspirates of bone marrow Free nuclei may be seen as well as small hyaline bodies that are undoubtedly part of the serum proteins being fragmented off from the ground substance Compressed erythrocytes can be seen squeezing their way through the sponge like mass of ground substance making up the splenic pulp The open circulation is apparent

Figure 18 presents photomicrographs from a case of systemic lupus erythematosus This and certain other mesenchymal diseases are pathologically so similar to Hodgkin s disease lymphomas and leukemias that I believe these too should be considered mesenchymal diseases

### *Mesenchymal diseases as diseases of environmental origin*

I also believe that all of the mesenchymal diseases are primarily environmental in origin An individual idiosyncrasy appears to be fundamental to the disease process and there is often a long latent period between exposure and appearance of the disease The precipitating factor may be infection or some similar aggravating incident occurring after the patient has withdrawn from contact with the intoxicating agent

Chronic poisoning with benzol affords a good example of disease caused by environmental factors Benzol is capable of producing





*Figu* 18 11 t m graphs of a pirat f ple ic p lp fr m a p t nt with ystem lupu ryth matos s. Compressed eryt ocytes may be see pa ing thro gh th int rsti es. U der re lu ed l ght (l er) th het g ity of the grou d bst e m y be te l and th hyal bod es so prom n nt n m ny of th lymph ma l leukem m y be seen

aplasia of the marrow hyperplasia of the marrow acute leukemia, chronic myelitis plenomegaly and according to Mallory, other conditions difficult to distinguish from neoplasia Mallory in fact, stated 20 years ago that biologically toxic agents can be roughly classified into two categories—substances that, in adequate dosage will produce their effect on all exposed individuals and substances that, in minute doses will produce in a few individuals devastating effects that cannot be duplicated in the majority of subjects even after administration of large doses of the agent

In leukemia lymphoma systemic lupus erythematosus and the other mesenchymal diseases, the history if carefully elicited is often significant in this connection Classic cases of systemic lupus erythematosus may occur in patients receiving hydralazine hydrochloride (Apresoline®) and may ubside when the drug is no longer given Similarly I have seen a number of women with systemic lupus erythematosus who gave histories compatible with sensitivity to permanent wave solutions An epidemic of aplastic anemia once was caused by use of a certain hair preparation

It is intriguing that in the e di ease there appears to be a synergistic effect of various agents — the association of x ray for example with infection or with administration of benzol compounds

## Discussion

Dr R A Good We have been interested in studying the reactivity of the individual to solvent materials of the sort that were mentioned You can readily sensitize the skin of the rabbit or human to benzol compounds The reaction produced can be classified with the delayed type of sen itivity In other words the e compounds produce reactivity in the human and the animal very much like that of the tuberculin reaction We have recently been able to transfer this en itivity using leukocytes of the peripheral blood and thus produced generalized allergic reactions

Dr Hargraves I would like to mention that most paint and varni h removers contain considerable amounts of benzene Hundreds of do it yourself people are being exposed daily in their own work hops I believe the hazard of such exposure is magnified by the increasing likelihood in our medical centers of exposure of the subject to x ray

# Dermatomyositis and Scleroderma in Childhood

DR RALPH J WEDGWOOD In reviewing the literature I have come to the conclusion that generalized scleroderma is one of the most rare of all the collagen diseases in childhood more rare than systemic lupus erythematosus, and probably at least as rare as polyarteritis nodosa

Dermatomyositis on the other hand is a relatively common disease More than 100 childhood cases have been described About 60% of the children with this disease survive Since their recovery is limited only by the degree of residual deformity early and correct diagnosis allowing early and adequate therapy should decrease the residual morbidity

## *Diagnosis of dermatomyositis*

Reliance on biopsy for pathologic diagnosis of dermatomyositis is in my experience unsatisfactory Not always do we see the classic changes in the muscle bundles with vacuolization centralization of nuclei degeneration infiltration between bundles and perivascular changes Early in the disease pathologic findings are usually only confirmatory not diagnostic

I should like to describe a few symptoms and signs in dermatomyositis which are so peculiar to that disease that they might constitute major criteria as for example a diastolic murmur in the aortic or mitral area can constitute a criterion for the diagnosis of rheumatic fever The frequency of the most important signs and symptoms of dermatomyositis are listed in figure 19 Four are of particular value in diagnosis weakness, induration of tissue facial lesion and pain and tenderness

The onset of the disease is extremely insidious and often the first sign is weakness or easy fatigability The weakness in children is usually first apparent in the legs, although involvement of the shoulder girdle often exists earlier and may be more severe Eventually the weakness and fatigability become so obvious that advice is sought Involvement of the muscles of respiration and of the palate are of particular importance Prior to the days of therapy with steroids almost all the fatalities occurred because of such palatorespiratory involvement

Induration of the tissue is more difficult to describe The involved muscles and subcutaneous tissues in dermatomyositis have a particular feeling on palpation variously described as thickened leathery indurated brawny rubbery or inelastic With satisfactory relaxation in the patient the muscles of the shoulder girdle particularly the superior portions of the trapezius in the neck are likely to demonstrate this phenomenon

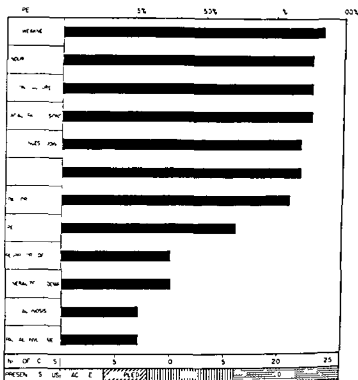



*Figure 19* The incidence of various signs and symptoms in children with dermatomyositis Reprinted from Pediatrics *12* 418 (October) 1953

The third important finding is the butterfly rash of the face which usually is no more than a slight erythema and induration with occasional scaling of the malar areas and the bridge of the nose It is rarely as severe as may be seen in systemic *lupus erythematosus* The butterfly distribution of the rash is less significant as a diagnostic sign than the violaceous heliotrope or faintly erythematous discoloration that becomes apparent on the upper lids of the eyes This

sign is usually present in dermatomyositis and occurs rarely if ever in other diseases with which it might be confused It may or may not be associated with periorbital edema

The child s pain in dermatomyositis is frequently localized by the parents to the joints The children themselves usually localize the pain in the muscles It occurs on movement, occasionally at rest and occurs at the tendinous area of the muscle rather than in the joint proper This finding allows differentiation from the arthralgia of the other mesenchymal diseases Tenderness may also be demonstrable in the muscle bundle itself These findings and the almost imperceptible onset of the disease differentiate it from viral myalgia or trichinosis

### *Manifestations in scleroderma*

In scleroderma hide binding might be considered one major diagnostic sign Others might be the stiffening of the fingers visceral changes particularly of the esophagus and cardiac findings which in adults are apparently more frequent in scleroderma than in dermatomyositis No data on the frequency of occurrence of these changes is available as far as I can find for the pediatric age group

### *Therapy in dermatomyositis*

Functional recovery from dermatomyositis depends on prevention of contractures and correction of the deformities that cannot be prevented To this end there is no substitute for vigorous physiotherapy planned carefully supervised well and made acceptable to parents and patient Physiotherapy should be instituted in spite of active disease Orthopedic assistance should be sought early to prevent contractures Early ambulation is probably the optimal form of therapy

Steroids have been used and with certain reservation have apparently aided therapy by providing symptomatic relief In our experience they have not altered the mortality In some of these patients the symptomatic relief is quite striking allowing early physiotherapy and early ambulation There is also a return of muscular function which may be due to a decrease in pain but probably also involves actual increase in muscular power

Prior to the availability of steroid children died mainly because of palatorespiratory involvement Of the children to whom we have given steroid six have died only one of palatorespiratory involvement alone Two died of respiratory involvement and another factor This factor probably the major cause of death in three other patients as well as a contributory cause in these two was a change in

the gastrointestinal tract Five of the six children suddenly developed gastrointestinal crises with perforation of unsuspected ulcer paralytic ileus and ga trointe tinal hemorrhage

Gastrointestinal lesions in dermatomyositis described prior to the availability of steroids do not seem to have been as severe as those seen in children who die while receiving steroid therapy These changes are probably an accentuation of the vascular changes seen elsewhere in the body in this disease and are probably intrinsic to the disease itself

## Nephrosis and Nephritis in Childhood

DR CAROLYN F PIEL Nephritis and nephrosis although not generalized do involve vascular mesenchymal tissue and hence war rant discussion with the mesenchymal diseases

### *General considerations*

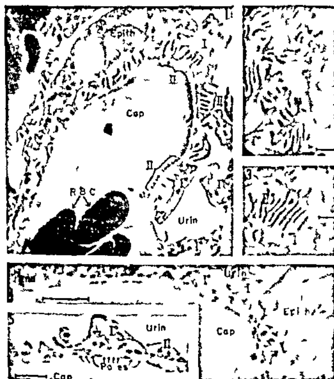
Acute glomerulonephritis and the nephrotic syndrome are gen erally recognized as distinct clinical entities in the pediatric age group The frequent occurrence of the nephrotic syndrome in patients with known chronic glomerulonephritis and the finding of histologic changes indistingui hable from those of chronic nephritis in the glomeruli of patients with nephrosis have led to the close association of the two illnesses in the minds of pathologists and investigators

The two diseases are differentiated by age of onset the inciting role of certain strains of beta hemolytic streptococcus in acute glom erulonephritis and the prominence in that disease of hematuria azo- temia and hypertension in contrast to the hypoproteinemia hyper lipemia ana arca and greater degree of proteinuria in nephrosis Renal clearances of inulin and para aminohippurate are often di minished in acute glomerulonephritis and tend to be normal or super normal in nephrosis In addition the prognosis is much more favor able in acute glomerulonephritis than in nephrosis

There are indications that acute glomerulonephritis is a disease of allergic origin These include a known specific inciting agent the beta hemolytic treptococcus the latent period of 10 to 14 days be tween the acute streptococcal infection and the onset of nephritis the excessive increase in titer of antistreptolysin O and the decreased titer of complement in the serum In addition glomerulonephritis comparable to the human disease can be produced in animals by various immunoallergic procedures

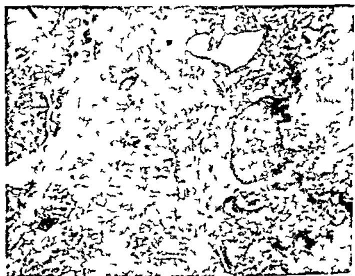
Nothing can be said about the period of latency for development of nephrosis since the inciting agent is not known. Most observers have found that the titer of antistreptolysin O is low in patients with nephrosis who are edematous. The suggestion that this low titer results from the loss of antistreptolysin O in the urine has not been confirmed.

The concentration of complement in the serum is reduced in both diseases, a finding interpreted by some as evidence that nephrosis is a disease of antigen-antibody reaction. However, determinations of the concentration of complement are not reliable in the presence of




*Figu e 20.* Low power lectron m r graph of gl m rul r p llary l m n ((ap) an l ur nary pa e (Urin) howi g ep th l al ll (Ep th) d it primary (I ) a l t rmin l (III ) e te o . The foot processes t digitake a seen in & 3 an l re separat d f m the l asement meml rane of th ap llary loop by em nt lst . Sect o 4 dem t ates th endothel l cell (End.) also separated f m basement m mbra l y e ment s lstan and 5 the sy t m of pores that t n erse the att u t d nd th l al heet. R print d from An t mi al Rec rd l l l 1955.





*Figu e 21* Flectron photomic ographs of the kidneys of an mals fed silver nitrate in the r drinking water for many months prior to experimentation (Upper) Section from a control an mal not treated with nephrotoxic serum The silver can be seen as black dots w thin the basement membrane A few tiny dots can be seen in Bowman s capsule and occasionally some can be seen in the b sement membrane of the tubules. (Lower) Section from an animal that had received nephrotoxic serum three days prev ously A prominent increase in epithelial substa ce may be noted as well as some thicken ng of the basement membrane The foot processes have almost lost their identity

lipemia and the possibility of loss of complement in the urine makes the evaluation of any change in concentration of complement difficult Two other findings are perhaps more sugge tive of an allergic ba is for nephrosis Localization of gamma globulin in the l asem nt m m brane of the glomeruli of patients with nephrosi ha been d mon strated and an experimental model of nephro is can l e j ro luced l y immunoallergic techniques

## *Experimental renal disease in animals*

Two types of nephrotoxic serum are u ed to produce experi mental disea e erum prepared l y injecting emul ion of kidney from another pecies into ducks or hen and erum prepared in rabbits It has been repeatedly demonstrated that the pecific antigen lod es in the bas ment membrane of the glomerulu It is thought to be protein bound with polysaccharide or lipid Nephrotoxic serum prepared in rabbit injected in adequate dose produces di ease immediately A depres ion of complement al o occur l ut i ap parently non pecific ince it occurs also in nephrectomized animals

We are currently engaged in tudying the glomerular changes in renal disea es in humans and in experimental animal as ol erved l y li ht and electron microscopy Figure 20 presents a micrograph of th glomerulus as seen with the electron microscope and figure 21 pre sents electron photomicrographs of the kidneys of animals fed silver nitrate in their drinking water for many month The changes seen three days after admini tration of nephrotoxic erum may l e com pared with the histologic appearance in the control animal The cement sul tance is lo t oon after a lmini tration of nephrotoxi serum l ut in the animal that have received silver nitrate cement sub tance cannot l e vi ualized even prior to treatment with nephro toxic serum Three week after admini tration of nephrotoxic serum the foot proce s of the epithelial cells are somew hat restored cem nt sul tance can be seen an l yet there per i t a thickening of the l a ment membrane

Electron microscopic examination of renal l iopsies of children with nephro is indicate that the alteration in the epithelial layer lo of identity of foot proc sses, absence of cement ul tance an l thicken ing of the basement meml rane parallel closely the chang s seen in the renal disease produce l in rats l y admini tration of nephrotoxi ral l it serum Som of these changes disapp ar with clinical r mi ion in luced l y steroi l therapy

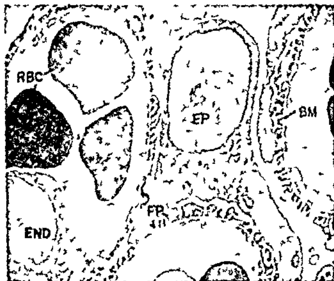
# Renal Biopsy in the Study of Chronic Renal Disease In Children

DR ROBERT L VERNIER We have examined by light microscopy specimens of kidney tissue obtained at laparotomy in 12 patients and by percutaneous needle aspiration in more than 90 other children with renal disease Electron microscopic study of approximately one half of these specimens has been completed in cooperation with Farquhar of the Department of Pathology University of California San Francisco From our experience it appears that 10 or more glomeruli and associated tubules are adequate for diagnosis in the group of diffuse renal diseases we have studied

The specimen is obtained under local anesthesia with the patient in the prone position The Franklin modification of the Vim Silverman needle is used The only complication we have encountered is the formation of a small perirenal hematoma in a three-year old child with the nephrotic syndrome There are no recognizable sequelae of this complication Although most children have microscopic hematuria for a short interval after this procedure only two instances of brief gross hematuria have been observed No significant alterations in the renal clearance of inulin have been measured when this study was performed before and after renal puncture The children tell me the procedure hurts less than aspiration of the bone marrow

## *Findings by light microscopy*

In our recent study of 25 cases of anaphylactoid purpura Addis counts indicated that 70% of the children had renal involvement We have studied renal specimens from seven of these children and have found that the renal lesion is characterized by proliferation of endothelial cell and focal fibrinoid occlusion of the glomerular capillaries There is tremendous variation in pathologic findings from patient to patient depending on the duration and severity of the disease at the time of the biopsy The morphology of the renal lesion in some patients resembles that seen in systemic lupus erythematosus.



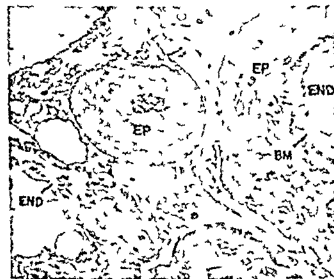
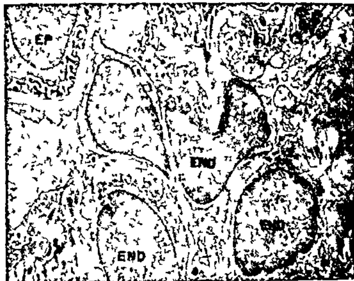




*Figure* [illegible]. Electron micrographs of sections of renal biopsies in children. (Upper) Normal glomerular loop demonstrating the epithelial cell (EP) with its foot processes (FP), the basement membrane (BM) and an endothelial cell with its attenuated cytoplasm (END). (Lower) Lesion seen in so-called pure nephrosis, demonstrating the striking loss of epithelial foot processes. The basement membrane is essentially normal but the endothelial cytoplasm appears swollen.







*Figu e 23* Electron micrograpl s of ect on of renal biop ie in ch l dren (Upper) Les ons seen n acute glomerulonephritis The mo t char acter tic find n s are a marked endothel al prol feration and a beg nnin accumulation of mat r l wh ch resembles basement membrane in density The epithelial foot processes are essentially normal (Lower) Les ons seen in chronic glomerulonephrit s The lumina of many of the glom rular loops have been liminated by endothelial proliferation and a umulati of basem nt m mb an like materi l

In systemic lupus erythematosus the renal involvement is often severe and is frequently the cause of death. The classic pathologic lesion is said to be the wire loop alteration of the arterioles. In our studies by light microscopy the renal lesion of systemic lupus erythematosus was often pathologically indistinguishable from the lesion of anaphylactoid purpura or glomerulonephritis in its various forms. In general there was considerable variation from minimal endothelial proliferation and focal glomerular occlusion by fibrinoid to severe hyalinization. No characteristic wire loops were seen. Some evidence of reversal of proliferative changes after treatment with cortisone was noted in one case.

## *Glomerulonephritis*

We studied a 12 year old boy 10 days after the onset of sore throat, fever and lymphadenopathy. Hypertension and hematuria were present and the beta hemolytic streptococcus type 12 was cultured from the throat. A clinical diagnosis of acute glomerulonephritis was made. There was no history of previous renal disease. On biopsy two types of lesions were found: one acute, compatible with the classic description of glomerulonephritis. There were large numbers of polymorphonuclear leukocytes in many of the glomeruli, striking proliferation of endothelial cells and glomerular ischemia. However chronic changes were also present, consisting of glomerular hyalinization, considerable tubular atrophy and interstitial infiltration. The chronic changes appeared to antedate the clinical history of onset by many weeks. One month later the proliferative changes had been ameliorated but the chronic changes persisted. The patient's course was that of chronic nephritis.

The pathologic findings indicated that this boy had unrecognized renal disease of insidious onset, and suffered an acute exacerbation through infection by this strain of streptococcus. Had the patient not been studied by biopsy the sequence of events in this case would have appeared to support the view that acute glomerulonephritis can progress to chronic glomerulonephritis. Perhaps some of the discrepancies in the literature regarding the relationship between these diseases will eventually be resolved by studies of this type.

I think that chronic glomerulonephritis is a pathologic end state the etiology of which we do not know; it may have several. It is probably no more specific a diagnosis than cirrhosis of the liver and indicates little of the nature of the inciting agent.

## *Nephrotic syndrome*

From our experience with the nephrotic syndrome we think that the renal lesions are related to both the severity and the duration

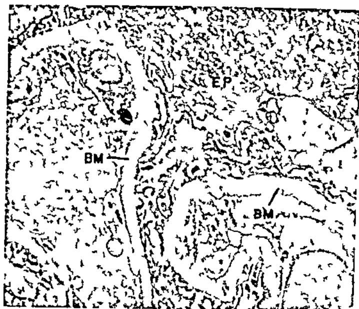



*Figure 24* Electron micro raph of a section of renal biopsy in a child Lesions seen in systemic lupus erythematosus The most striking abnormality present early in the disease is a general zed thickening of the basement membrane proper Here the ba ement membrane is four to six t mes normal thickness

of the disea e Early in the course of the disease the kidney appears normal by light microscopy If the disease has persisted for many months there is likely to be considerable scarring with many hyalin ized nonfunctioning glomeruli despite the absence of clinical and laboratory evidence of nephritis

At times the renal lesions of children with nephrosis with sub acute glomerulonephritis and with systemic lupus erythematosus may be strikingly similar by light microscopy We have utilized the electron microscope in the study of the early ultramicroscopic morpho logic alterations that occur in the various diffuse renal disea es

## *Findings by electron microscopy*

With the electron microscope three basic components of the glomerular capillary—epithelium basement membrane and endothe lium—are readily seen The e three distinct membrane layers are shown to be differentially involved in nephro is glomerulonephriti and the nephritis of sy temic lupus erythematosus

The ultramicroscopic pathology of the nephrotic syndrome is characterized by distortion and smudging of the foot processes of the epithelial cell. The basement membrane proper and endothelium remain essentially unaltered early in the disease. This marked alteration of the epithelial cell is present regardless of the clinical state of the disease or the severity of change as seen by light microscopy.

In glomerulonephritis, the endothelial cells undergo marked proliferation and variable quantities of a material which resembles basement membrane appear in intimate relationship to the cytoplasm of the endothelial cells. The epithelial cells are normal except in very severely involved glomeruli.

Systemic lupus erythematosus is associated with a renal lesion characterized by thickening of the basement membrane proper. The endothelium and epithelium are relatively normal and become significantly distorted only late in the process.

## Discussion

DR EHRICH: In animals it is possible to study the pathogenesis of these various lesions step by step under controlled conditions. On the basis of such studies I should like to offer an alternative interpretation of the changes demonstrated by Vernier. I believe that many of the lesions shown to us were complications of the underlying disease consisting of thrombosis of glomerular loops and eventual reorganization of thrombotic material. In many cases the thrombosis had led to leakage of fibrinoid exudate into the capsular space which became organized with the formation of crescents. These complications are seen in all glomerular diseases with the exception of amyloidosis. We see them in systemic lupus erythematosus, in glomerulonephritis and in nephrosis.

DR VERNIER: I fully agree that the thrombotic manifestation seen in light microscopy may be a complication of the basic disease process. I do not believe I can separate the complications from the disease. However, the three basic components of the glomerular capillary, as seen by electron microscopy, are differentially involved in the three diseases. Although the changes may be somewhat sparse or varied from one glomerulus to the next, changes in the endothelium, epithelium and/or basement membrane make it possible to distinguish the various pathologic processes.

All our current conclusions should certainly be considered tentative. But I think these changes indicate that the renal expression of a disease may depend on the particular component of tissue involved early in the pathogenesis of the disease. The injury to the

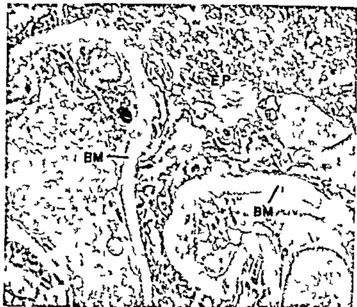



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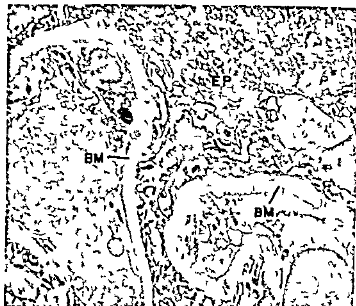



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epithelium per se results in massive proteinuria and development of the nephrotic syndrome injury to endothelial cell results in gradual development of glomerulosclerosis, hypertension et cetera

DR R A GOOD Experimental models are not always adequate for the purpose Sometimes relying on experiments of nature provides an even more incisive approach than does experimentation with animals We have had an unique opportunity to study an interesting experiment of nature in which four children in one family developed the nephrotic syndrome early in life From the clinical point of view the diseases that these children presented at the time we studied them were respectively transient pure nephrosis, persistent pure nephrosis mixed nephrosis and nephritis and chronic glomerulonephritis We can assume although there might be some doubt about the validity of the assumption that these children all suffered from the same basic etiologic process

Pathologically using the light microscope the entire clinical spectrum of renal disease was revealed The two children with pure nephrosis had no demonstrable renal lesion The child with mixed nephrosis and nephritis showed pathologic evidence of proliferative glomerulonephritis The other child pathologically as well as clinically had typical chronic glomerulonephritis

All of these kidneys when studied by the electron microscope showed the same basic lesion namely the smudging of the foot processes along the basement membrane proper that Vernier has illustrated We have been forced from this observation to conclude that the several forms of the nephrotic syndrome diagnosed by clinical criteria and pathologic changes seen by light microscopy are in reality manifestations of a single basic disease process an early concomitant of which is the disturbance in morphology of the epithelioid cell podocytes that has been revealed by electron microscopy

## Anaphylactoid Purpura in Children

DR THEODORE C PANOS My purpose is to review the syndrome of anaphylactoid purpura to present some pertinent case material and to discuss the similarities between this disease and other mesenchymal diseases

### *Designations and major manifestations*

The disease is designated by a variety of names Henoch Schonlein syndrome is preferred by Gairdner allergic purpura is preferred

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The di ea e is a polymorphic or protean recurrent di ease du t hypersen itivity and resulting in a generalized di turbance of n all vessels It is characterized by the following major manifestat occurring in any combination or equence a macular papular n l carial petechial or purpuric ra h ga trointestional s n and y tems including colic vomiting melena diarrhea and intu usc p and involvement of joints manife ted by pain and sy ellin It h frequently been tated that the pre ence of any two major manifesta tions con titutes a ba is for po itive diagno i Fever when pres nt is u ually low grade Renal involvement is often con picuous a d varies from microscopic hematuria to gro s hematuria with hy tension azotemia and uremia

In ord r to delineate more atisfactorily the relation hips an the various manife tation we undertook an analy is of 572 c curring in children less than 15 years of age and reported in th En lish language during the pa t 20 year Well over 90% of these cases and two thirds of the publications have appeared sinc 1951 The incid nce of the disease has definitely increa d durin re year

## *Frequency of various symptoms and sign*

The sex di tribution is 3 to 2 with predominance in males The age at onset ranges from 8 months to 15 years the hi he t inci l nce occurring between 3 and 12 year with the median and mean at about 5 year The duration of the acute pha e is from 6 to 180 days for the great majority of cases with a median of about 21 day At l a t one recurrence can be expected in about one third of th cases One half of the recurrences were in le and mo t took place within 2 to 12 months of th first attack Only rarely did the interval exceed six month although occa ionally a much as two year elapsed between episode Infections are very often cite l as precipitating fa tor in relapse The mortality i about 2% mainly from r nal failur

Individual case hi tori s were ufficiently detailed in 89 in tances to allow the tabulation of frequen y of occurrence of certain signs, symptom and antecedent circum tances Antecedent infection oc curred in 33 cases or 37% Thirty two of these infecti n were of the upper respiratory tract, and beta hemolytic streptococcus wa cultured from nearly half of the pati nts. The most commonly tated interval between inf tion and onset of disease wa 7 to 14 days A nine year

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The disease is designated by a variety of names Henoch Schonlein syndrome is preferred by Gairdner allergic purpura is preferred

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The disease is a polymorphic or protean recurrent disease due to hypersensitivity and resulting in a generalized disturbance of small vessels. It is characterized by the following major manifestations occurring in any combination or sequence: a macular papular urticarial petechial or purpuric rash; gastrointestional signs and symptoms, including colic, vomiting, melena, diarrhea and intussusception; and involvement of joints manifested by pain and swelling. It has frequently been stated that the presence of any two major manifestations constitutes a basis for positive diagnosis. Fever, when present, is usually low grade. Renal involvement is often conspicuous and varies from microscopic hematuria to gross hematuria with hypertension, azotemia and uremia.

In order to delineate more satisfactorily the relationships among the various manifestations, we undertook an analysis of 572 cases occurring in children less than 15 years of age and reported in the English language during the past 20 years. Well over 90% of these cases and two thirds of the publications have appeared since 1951. The incidence of the disease has definitely increased during recent years.

## *Frequency of various symptoms and signs*

The sex distribution is 3 to 2, with predominance in males. The age at onset ranges from 8 months to 15 years, the highest incidence occurring between 3 and 12 years, with the median and mean at about 5 years. The duration of the acute phase is from 6 to 180 days for the great majority of cases, with a median of about 21 days. At least one recurrence can be expected in about one third of the cases. One half of the recurrences were single and most took place within 2 to 12 months of the first attack. Only rarely did the interval exceed six months, although occasionally as much as two years elapsed between episodes. Infections are very often cited as precipitating factors in relapse. The mortality is about 2%, mainly from renal failure.

Individual case histories were sufficiently detailed in 89 instances to allow the tabulation of frequency of occurrence of certain signs, symptoms and antecedent circumstances. Antecedent infection occurred in 33 cases, or 37%. Thirty two of these infections were of the upper respiratory tract, and beta hemolytic streptococcus was cultured from nearly half of the patients. The most commonly stated interval between infection and onset of disease was 7 to 14 days. A nine year

study by Lewis of 139 cases of anaphylactoid purpura 290 of glomerulonephritis and 297 of rheumatic fever elicited comparable incidence of antecedent upper respiratory infection—50% 46% and 64% respectively He was able to culture hemolytic streptococcus from one fourth of the patients in each group

Past history of allergy was elicited in 8% of the 89 cases, family history of allergy in 13% an incidence not significantly greater than in the general population Foods were specifically implicated as an inciting factor in 11 cases The most common items mentioned were chocolate egg albumin tomatoes pork and fish Insect bites were implicated in three cases Drugs were specifically mentioned only in two cases in each case penicillin

The frequency of signs and symptoms in these cases listed according to systems is given in figure 25 Forty eight were described in sufficient detail to allow a tracing of the sequence of appearance of signs and symptoms In decreasing order the most common initial complaints were colic purpura swelling of the joints vomiting pain in the joints and petechiae Fever was not a prominent early finding

Renal involvement deserves special comment because of its frequency and because it is the most common cause of death Hematuria appeared within the first week in two thirds of the cases Azotemia and/or hypertension occurred in 40 to 50% of the patients with gross hematuria, a finding present in one fourth of all patients with renal involvement Although the vast majority of patients recovered promptly there was a tendency in some for microscopic hematuria to persist from several months to several years perhaps indicating latent or chronic nephritis

It was possible to find reports of treatment with either adrenocorticotropin or cortisone in only 42 cases In 14 the results were described as excellent in 10 as fair and in 8 as poor Exacerbation after withdrawal of steroid therapy occurred in 17 patients and 10 required retreatment In patients with renal involvement the steroids were conspicuously ineffective

## *Relationship between anaphylactoid purpura and polyarteritis nodosa*

Ever since Osler in 1914 drew the analogy between serum sickness and anaphylactoid purpura and predicted that before long the anaphylactic key would unlock the mystery of these cases tremendous interest has been generated in the relationship of the anaphylactoid purpura syndrome to mesenchymal diseases Similarities between anaphylactoid purpura nephritis rheumatic fever and polyarteritis nodosa were recognized early as was the fact that differentiation among these entities is occasionally virtually impossible at certain

stages of their development Gairdner in 1948 grouped anaphylactoid purpura with rheumatic fever nephritis and polyarteritis nodosa to form a family of diseases that he considered to be linked together by the tendency for one to coexist with another and by characteristics common to the group He also contended that anaphylactoid purpura and polyarteritis nodosa are essentially the same disease process capillaries being involved in the former medium sized arteries in the latter with arteriolar involvement common to both

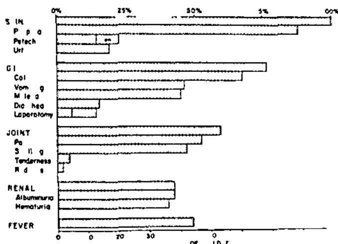



*Figure 25* The frequency of various signs and symptoms in anaphylactoid purpura in children

Actually there is a surprising paucity of pathologic material on anaphylactoid purpura and most of the evidence linking this disease with polyarteritis nodosa is circumstantial Even histologic studies, although strongly suggestive are not conclusive

However the following similarities do provide evidence of an interrelationship both are generalized vascular disorders with aseptic inflammation of the vessels of skin gastrointestinal tract joints and kidney and this vascular involvement is sufficient to explain the clinical picture Necrotizing arteriolitis occurs in severe forms of anaphylactoid purpura Both are related to antecedent infection in a manner suggesting that hypersensitivity plays a role Also in adults, the progression of anaphylactoid purpura into classic polyarteritis nodosa has been reported Polyarteritis nodosa can readily be produced by injections of horse serum and at least the major manifestation of anaphylactoid purpura can be reproduced in the same manner

Arguments can be raised against the likelihood that anaphylactoid purpura and polyarteritis nodosa are closely related diseases The lesions are not identical In anaphylactoid purpura there is an infiltration about the capillaries rarely about arterioles and then only in the very severe and/or fatal cases Polyarteritis nodosa begins with and is characterized by segmental necrosis of the medium sized arteries and there is a paucity of lesions in capillaries and veins Nodosa lesions have not been reported in anaphylactoid purpura The rarity of cardiac lesions in this disease contrasts with the incidence of 84% in polyarteritis nodosa Renal involvement is much more common in polyarteritis (75%) than in anaphylactoid purpura (35 40%) The reversibility of the lesion of anaphylactoid purpura is in contrast to usual irreversibility in polyarteritis nodosa Polyarteritis is ordinarily fatal whereas anaphylactoid purpura is rarely fatal However it seems to me there is no doubt that anaphylactoid purpura belongs to the family of mesenchymal diseases

## Hormone Therapy in Rheumatic Fever

DR ALAN K DONE While there is general agreement that administration of hormonal agents and salicylates produces more or less prompt subsidence of the acute symptoms of rheumatic fever the opinion that none of them alters the frequency of cardiac sequelae has been advanced repeatedly These conclusions have been based on data collected during studies with a limited variety of therapeutic regimens very few of which were individualized in any way There would seem to be little reason to believe that adrenocorticotropin and cortisone in contrast to other medications can be used without regard for response of the condition under treatment

### *Individualization of hormonal therapy*

In our studies individualization of therapy was accomplished by 1) adjusting the size of the dose to the size of the patient that is 1 I U of adrenocorticotropin or 3 mg of cortisone per pound of body weight per day as the minimum initial dose 2) adjusting the size of the dose to the severity of the illness severely ill patients receiving a larger initial daily dose and 3) adjusting the duration of therapy according to the response of the patient Therapy with the full initial dose is continued not for a fixed period of time but until there is no longer any clinical and laboratory evidence of activity of

the disease In patients treated early during their attacks of rheumati fever this initial maximal dosage is usually given for two to four weeks

Because the clinical signs of disease disappear rapidly under the influence of hormones it is difficult to arrive at a sati factory d fini tion of the proper duration of therapy with the initial maximal dosa The erythrocyte sedimentation rate and concentration of C rea tiv protein in the serum are not satisfactory criteria ince these valu become normal too soon after the initiation of therapy Di ontin u tion of treatment at that point is likely to re ult in clinical relap

There is little hazard of clinical relapse if hormonal th rapy i continued in maximal do age until the concentration of mucoprotein in the erum has decrea ed to le s than 6 mg/100 ml and if tapering of the dosa e is done gradually and cautiou ly In our clinic therap with the full initial dose is continued until the following criteria hav been met all clinical evidence of activity has di appeared the erythr cyte sedimentation rate has been normal for at lea t one week and th concentration of mucoprotein in the serum has decrea ed to 6 m 100 ml The dosage is then gradually reduced at intervals of two to three days Before each reduction of dosage the erythrocyte sedimentati n rate is determined If there is any evidence of relapse the dosa not further decrea ed until this evidence has di appeared

## *Effect of hormonal therapy on incidenc of residual cardiac damage*

The measure of efficacy of any treatment for rheumatic fever must be based princi ally on the production of a lower incidence of residual cardiac damage Unfortunately this effect is the mo t difficult to a ess In patients who have u tained heart damage from previou rheumatic fever or in whom the prior cardiac tatus is unknown such evaluation is nearly impos ible

A compari on has been made of the inci lence of resi lual cardiac murmurs in 85 patients who were known to have no murmur before the onset of an attack of acute rheumatic fever and who ha l exhibite l signs or symptoms of rheumatic fever for les than three month Of these 49 were treated either with adrenocorticotropin or cortisone an l 36 with salicylates or bed rest alone All these patients had un equivocal evidence of carditis prior to therapy Patients who ex hibited evidence of recurrence of rheumatic fever were incl ided in the follow up tu ly only to the time of the la t examination prior to the recurrence The findings in hormone treat d patients are grouped and compared with those treated with salicylates or bed rest alone since no significant difference in incidence of resi lual car liac mur murs was found between the groups treated with a lrenocorticotrop n

or corn one or between those treated with salicylates or merely bed rest

*Table 6* presents data on the incidence of diastolic murmurs or systolic murmurs of grade 2 or greater intensity at intervals after cessation of therapy Among the patients not treated with hormones the percentage exhibiting diastolic murmurs or systolic murmurs of grade 2 or greater intensity ranged from 42 to 65 throughout the four years of follow up In contrast among the patients treated with hormones the incidence declined to 9% within three months after discharge and at four years was 6% The results of application of a similar regimen of hormonal therapy in cases of recurrent and chronic rheumatic fever have likewise been encouraging Not only did fewer murmurs persist among the hormone treated patients but these individuals developed new murmurs during the follow up period far less frequently than did patients treated with salicylates or merely bed rest

Table 8

*The incidence of diastolic murmurs and of systolic murmu s of grade 2 or greater intensity at intervals after a first attack of acute rheumatic carditis*

| | Ac te Ph | At D s ch ge | 3 mo | 6 mo | 1 yr | 2 yr | 3 y | 4 yr |
|---|---|---|---|---|---|---|---|---|
| Hormone treated | | | | | | | | |
| % with murmur | 9? | 2? | 9 | 5 | 7 | 6 | 4 | 6 |
| Treated with salicylates or bed rest alone | | | | | | | | |
| % with murmur | 95 | 50 | 58 | 42 | 45 | 56 | 65 | 60 |

## *Adverse effects of hormonal therapy*

Among patients with rheumatic fever whom we have treated with hormones during the past five and one half years the only apparent adverse effects of therapy have been the temporary occurrence of facial changes suggesting Cushing s syndrome excessive appetite and the appearance or aggravation of acne In a few instances girls have developed urinary tract infections and a number of patients have developed paronychia These infections have not seemed inordinately severe and have responded readily to administration of antibiotics or chemotherapeutic agents Significant hypertension has not developed although we have observed it in younger patients who have received

larger doses of hormones in the treatment of hematologic disorders, and in a number of patients with renal disease In no patient with rheumatic fever have we found it necessary to discontinue the therapy because of side effects

These observations suggest that it is possible to treat most patients with rheumatic fever with sufficient doses of adrenocorticotropin or cortisone to achieve maximal benefit without encountering serious complications This does not imply that serious complications should not be anticipated and it should be borne in mind that similar doses of hormones have resulted in serious complication when used in treatment of diseases other than rheumatic fever It is our impression that the nature of the patient's illness is an important factor in determining what dosage of hormone will be tolerated

Among the patients treated with adrenocorticotropin or salicylates there appeared to be no significant reduction in responsiveness of the adrenal gland to administration of adrenocorticotropin after cessation of therapy Significant depression of adrenocortical response did occur after therapy with cortisone but persisted for less than one week

## Discussion

Dr Leonard M Lindy (Los Angeles California) How do you account for the lack of mortality in both the treated and untreated patients?

*Dr Done* In the group of patients treated with salicylates, there was one death 14 months after cessation of therapy The fact that none of the patients had previous cardiac damage is probably responsible for the low mortality rate As you know the mortality rate in first attacks of rheumatic fever and in recurrent attacks where previous damage to the heart has not occurred is relatively low

Dr Smith What was the method of selection of patients for inclusion in the various groups?

Dr Done Patients are currently selected at random for inclusion in the various groups Initially this was impossible because a significant percentage of patients was referred specifically for hormonal therapy Because most of the patients treated with hormones in the early part of the study were referred the severity of the illness was probably greater in this group making the results perhaps more significant

Dr Dorfman I am quite convinced from what we have observed that it is erroneous to assume that the patients who received salicylates can be considered an untreated control group Salicylates

have a rather marked effect on certain manifestations of rheumatic fever although I do not know whether they affect carditis

I agree that treatment with a fixed regimen has real disadvantages I would however point out that in the published data of Done and Kelley the average amount of drug given was similar to that used in the Cooperative Study

DR DONE It is true that the majority of patients in the Cooperative Study received the same total dose of hormones as did our patients I think more important than the total dose however is the dosage level at which these patients were treated relative to the duration of their disease It is important that the initial dose be sufficient to produce the desired response and that dosage be continued at that level until all evident disease activity has subsided Tapering the dosage gradually with the guidance of the clinical course and laboratory studies was probably a significant factor tapering was stopped at any point where clinical or laboratory evidence of relapse was noted

DR R A GOOD We do not see patients with rheumatic fever as early as Kelley Done and their coworkers do I presume this is largely a function of the geographic location and the relationship of the general physician to the medical center Although we have not studied a large series I think negative observations drawn even from relatively few patients have pertinence For example we have observed the development of new murmurs and have watched progression of rheumatic fever to fatal termination with overwhelming rheumatic carditis even in patients receiving large doses of adrenocorticotropin or cortisone We are not at present convinced that the carditis can be terminated or uniformly favorably affected by treatment with adrenal steroids

I am cognizant of the fact that data are accumulating in the literature supporting the position taken by Done and Kelley and their group which indicate that if these patients are treated early with enough hormone more is accomplished in treatment of carditis than was accomplished in the Cooperative Study or in most of the patients that we have seen at Minnesota

I think it unusual that Done using the dosages described has not observed serious untoward reactions to these hormones in patients with rheumatic fever Certainly other investigators have observed such complications (Dorfman et al ) We have observed severe infections psychoses of the most malignant type convulsions ulcers and severe damage to blood vessels and connective tissue in subjects with rheumatic fever who were treated with large doses of hormone The recent observations of Eley in Boston that several children under treatment with steroids in moderately large doses succumbed to infection with chickenpox needs only to be mentioned to warn us that

in patients with rheumatic fever as well as those with other diseases the use of these hormones may be hazardous

DR KELLEY Good implied that the regimen of hormonal therapy that he uses is the same as ours However in our regimen whenever a patient s condition is becoming worse we increase the dosage of hormone When a patient is critically ill we administer cortisol intravenously to no limit except improvement of the patient

Since we started this policy we have never had a death result from rheumatic fever in our hospital Previously there had been no year without such deaths in the records of our hospital Ours is an area of high incidence of severe rheumatic fever and this improvement occurred despite the increased number of cases seen since the institution of our present program

## Serious Untoward Effects of Hormone Therapy

DR ROBERT A GOOD It has become increasingly apparent that adrenocorticotropin and cortisone are extraordinarily potent pharmacologic agents, affecting mammalian physiology in several different ways It is being realized gradually that their effects are not all beneficial Many of the biologic actions of adrenocorticotropin and cortisone reproducible in the laboratory might be expected to present extreme hazards applied to human beings Both adrenocorticotropin and cortisone must be looked upon as two-edged swords capable of doing much harm if wielded indiscriminately

The overenthusiasm of physicians regarding the usefulness of these compounds is best exemplified by the popular practice of reporting in great detail the beneficial effects of their clinical utilization Description of consequences that are often life threatening or even fatal are relegated to the final paragraph or a footnote Few papers are devoted to the description and discussion of untoward reactions to adrenocorticotropin and cortisone while literally hundreds extol their virtues

### *Serious consequences of therapy in 35 of 310 children treated*

During the past three years we have used adrenocorticotropin and cortisone systemically in the treatment of 310 children nearly

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### *Serious consequences of therapy in 35 of 340 children treated*

During the past three years we have used adrenocorticotropin and cortisone systemically in the treatment of 340 children nearly

## Table 9

*Serious complications of administration of adrenocorticotropin and cortisone to children: disturbances of equilibrium of fluids and electrolytes and disturbances of the central nervous system*

| Patient | Complication of Therapy | Disease Under Treatment | Hormone Implicated | Dosage Used mg/m²/day | Duration of Treatment (days) |
|---|---|---|---|---|---|
| | Disturbances in equilibrium of fluids and electrolytes | | | | |
| D N, 4 yr, male | Pulmonary edema associated with generalized retention of fluid | Rheumatoid arthritis | ACTH | 150 | 21 |
| R L, 8 yr, male | Severe generalized edema, ascites and hypertension | Dermatomyositis | Delta cortisone | 75 | 21 |
| R S, 9 yr, male | Hypopotassemic shock after diuresis | Nephrotic syndrome | ACTH | 130 | 14 |
| N S, 4 yr, female | Hypopotassemic shock after diuresis during ACTH treatment | Nephrotic syndrome | ACTH | 130 | 14 |
| T G, 2 yr, male | Hypopotassemic shock after diuresis during ACTH treatment | Nephrotic syndrome | Delta cortisone | 75 | 14 |
| | Disturbances of the central nervous system | | | | |
| E R, 13 yr, female | Central retinal artery thrombosis, cavernous sinus thrombosis, blindness in right eye | Rheumatoid arthritis | ACTH | 77 | 14 |
| L H, 16 yr, female | Paranoid schizophrenia | Plasma cell hepatitis | Cortisone | 37.5 | 30 |
| S O, 13 yr, female | Paranoid schizophrenia | Rheumatoid arthritis | ACTH | 100 | 60 |
| D B, 8 yr, male | Status epilepticus with focal seizures and generalized seizures | Acquired hemolytic anemia with positive Coombs test | Cortisone | 75 | 330 |
| T R, 14 yr, male | Hypertensive encephalopathy, status epilepticus | Systemic lupus erythematosus | Cortisone | 300 | 30 |
| L H, 6 yr, female | Hypertensive encephalopathy, status epilepticus, residual transient paralysis | Leukemia | Cortisone | 1[illegible]5 | 5 |
| N M, 13 yr, female | Retention of sodium, edema, hypertension and status epilepticus | Subacute nephritis | Cortisone | 300 | 5 |
| S W, 6 yr, female | Hypertensive encephalopathy, status epilepticus, coma, stupor, aphasia | Acrodermatitis chronica mutilans | ACTH | [illegible]00 | 5 |

## Table 10

*S u. complicati ns of adm istrati n of ad enocort cot of n and c rt s ne t hild en the occu ence of infect us diseas and f gast o nt t nal d sturb nces*

| P tient | Com lica io f The y | Disease Under Trea | Hormone Im l cated | Dosag mg/ m /da | D ra f Trea men (days) |
|---|---|---|---|---|---|
| | | I fect D se | | | |
| J B 5 y mal | St phyl coc l sep m | Stev s-J h so y d om | ACTH Hyd rtison | 83 114 | 5 |
| M C 5 y f mal | Cell l t f k d bc t neo t f gh l g | N phrot drome | Del rt so | 5 | |
| L L 4 y f m l | H rpe zost | N ph ti sy d me | ACTH | 5 | 60 |
| W B 8 y m l | H rpes ost | Rheum t f | Delt rt sone | 71 | 60 |
| T B 2½ y m l | A t bd m l w th te l pe t tis d a t t D l hem h ge | Nephrot y d me | Delt rt sone | 100 | 14 |
| S V 13 y f m l | S l t p eumo m l pl bsces et t phylococ l sept m Aspe g ll p mo | Systemic L pu ryth matosus | ACTH CTH | 66 6 t l 82 2 | 30 |
| S W 6 y fem l | S hyl coc l ( l f aht l g | A rod rmatiti hro m t l | AC H | 150 | 5 |
| T K 5 y m l | Staph lococ l bscesses d sept m | Leuk m | Cort so | 00 | 30 |
| I T 8 y m l | Strept coc l mpy ma, me g ti sept c rth | Ot t med t t p | C rt so | 100 | 8 |
| | | Ga tro t st l D ord | | | |
| T P y m l | C tro test l hem rrh ge | N phrot yndrome | Del rt sone | 5 | 10 |
| T G 2 y m l | E g g m tes l bleed g | N phrot yndrome | Delt rt sone | | 10 |
| P T 12 y f m le | Perf n of leum h on | Nephro yndrome | AC H | 83 | 0 |
| S C 8 y m l | Perf rated ic w h sev ha l pe on t | Nephrot y drome | AC | 100 | 10 |
| R A y mal | P f t of lon & false st ph l c l bscesses d pneumon | Ul t ve c l | Cort one | 300 | 1 |
| W K 11 m l | B ee l pept l w h perf ration splenic ders | P lyarte ti th d sa | Cortisone | 66 2 0 | 170 |
| B M 15 y ial | P rf ed pept l | N hrotic drome | AC H | 83 | 1 |

CTH I U./m² day

all suffering from serious diseases In some instances there seemed to be little doubt that the underlying disease contributed to the untoward reaction observed However in selecting cases to discuss here I have chosen only those in which we felt that hormonal treatment was a major contributing factor and not a coincident event

Serious consequences of treatment of sufficient everity to threaten the life of the child were encountered in 35 of the 340 children an incidence of approximately 10%

Administration of adrenocorticotropin and corti one may produce retention of sodium chloride and water with marked gain in weight anasarca ascites pleural effusion and pulmonary edema (table 9) Depletion of potassium may produce abdominal distention abdominal cramps weakness listlessness and lethargy In several in tances we noted it to be associated with shock It is extremely difficult to determine the requirements of these patients for potassium In several instances in which we have administered relatively large doses of potas ium chloride the concentration of potassium in the serum has increa ed during the early pha e of treatment to rather alarming values

As indicated in the table convulsive seizures have occurred both associated with hyperten ion and in its absence Patients with pre existing renal disease are particularly prone to develop hypertension Other serious complications relating to the central nervous system include status epilepticus and paranoid schizophrenia

Probably the most frequent of the serious complications we have observed in the use of steroids has been the occurrence of severe life threatening infections (table 10) These have been particularly hazardous because they are often silent until far advanced The most dramatic complications have involved the gastrointestinal tract Among other complications are those involving the keletal system Generalized osteoporosis occa ionally with pathologic fracture is particularly likely to develop in patients with rheumatoid arthritis who receive prolonged steroid therapy

### *Potential dangers from use of steroid hormones*

There are ome serious potential dangers from the use of steroid hormones In experimental work enhancement of the effectiveness of oncotic agents has been observed as well as succesful transplantation of tumors in animals in which tran plantation is otherwise unsucce sful and metastasis of tumors that otherwise will not metastasize It is at least remotely pos ible that such effects of these extraordinarily potent pharmacologic agents may be manifest at a later date in humans

In Cushing s disease there is early and often dramatic development of arteriosclerotic complications In experimental animals

and in humans a dramatic effect on the concentration of lipids in the blood is observed The possibility of an adverse effect on the ultimate integrity of the vascular system in patients receiving long term treatment might therefore be considered

Typical rheumatoid arthritis of long standing and scleroderma have apparently been transformed into polyarteritis nodosa during treatment with steroid hormones

## *Poststeroid syndrome*

In long term therapy with large doses of delta steroid u in approximately 100 mg/m in the treatment of patients with rheumatic fever serious untoward reactions developed and the dosage was gradually decreased over a period of about 80 days These patients have regularly developed what we term "post teroid syndrom characterized by fever pruritus painful nodules fat necrosis and ometime elevated erythrocyte sedimentation rate and cardiac failure without evidence of reactivation of the rheumatic process We thought this might be a consequence of the large dosage of hormone employed but have learned recently that other investigators have observed similar phenomena after prolonged treatment with lower doses of delta cortisone Indeed Thomas Good and his coworkers have produced extraordinary manifestations in joints and muscles after a single large dose of delta steroid hormone

A significant danger exists when adrenocorticotropin and cortisone or its analogs are employed in clinical pediatric practice The danger is greatest when large doses of the hormone are used and prolonged treatment employed but is expressed in some patient a the result of treatment in relatively small dosage over a hort period of time These hormones are also potentially hazardous in ways in which clinical expression of the hazard has not been clearly established

We are forced to recognize that in children as in adults, these drugs are extremely potent and dangerous pharmacologic agents of which we have incomplete understanding In our opinion their use should be reserved for circumstances of real peril consisting of threat of death threat of prolonged permanent incapacitation or threat of irreversible damage to vital tissues and organs

## Discussion

Dr Done Since we have not seen serious complications in patients with rheumatic fever although we have seen them at times

in other illnesses it was interesting that Good rarely referred to complications of therapy in patients with rheumatic fever I wonder if he believes that the underlying disease is as important a factor as we do in deciding whether the patient is likely to develop serious complications while receiving a particular dosage

The term po tsteroid syndrome seems too broad since the phenomenon was observed only in patients who had received the delta steroids and not in patients who had received cortisone or adrenocorticotropin I do not think there is evidence indicating that administration of other steroids is followed by imilar complications

Dr R A Good I do not know why Done s patients with rheumatic fever appeared to be somewhat immune' to these complications perhaps tho e we ee differ from those he sees Three thousand cases of rheumatic fever were reported in Minnesota last year but the great majority of patients presumably with relatively mild disease were cared for by their family physicians In the few patients referred to us because of rheumatic fever there has been a high mortality despite steroid therapy perhaps then there is a difference in the form of the disease Done has been studying However we have certainly ob erved threatening consequences of steroid therapy in some of our rheumatic children Fluid retention wor ening of heart failure frank psychosis of a paranoid schizophrenic type convulsions and gastrointestinal ulceration have all occurred

I agree that the poststeroid syndrome may apply only to treatment with specific steroids perhaps the delta steroids Although we have found that it is generally difficult to admini ter effective doses of the delta steroids without production of serious reactions dosage undoubtedly is a most important factor In experimental work with rabbits we have observed a poststeroid usceptibility to endotoxin which follows administration of either cortisone or the delta steroid

Dr C Henry Kempe From the data presented the dosage of hormone appears to be a more significant factor in promotion of bacterial infection than does the duration of treatment

Dr R A Good Our experience indicates that it does not take very long to develop susceptibility to infection even when administering only moderate doses

Dr van Creveld We have studied a child who has had diabetes mellitus for four years following treatment of nephrotic nephritis with moderate doses of steroids This steroid diabetes is characterized by a light tendency to ketosis relative insensitivity to insulin and marked decrease of glycosuria when the intake of protein is decreased

## Summary

CHAIRMAN VINCENT C KELLEY Although much of the data presented during this conference cannot be interpreted with our present understanding of the illnesses under consideration there have been many significant contributions that will clarify our perception Investigations of chemical and physiologic properties of beta heparin and of fibrinogen and related proteins may well point the way to a clearer understanding of the functions of connective tissue and its alterations in mesenchymal diseases The phenomenon of micellophagosis and the recognition of dissociant forms of bacteria and their possible significance in pathogenesis of rheumatoid arthritis are of great interest The demonstration of altered patterns of adrenal steroids in the plasma of patients with rheumatic fever and in unaffected siblings of these patients suggests that alteration of adrenal function occurs in rheumatic fever and may precede rather than result from the disease Discussions of the individual mesenchymal diseases have been illuminating and the serious consequences as well as beneficial effects of therapy with steroid hormones have been vividly portrayed

It is my hope that all of the investigators who have met here will have been stimulated by the exchange of ideas to return to their investigations with renewed vigor